Pupil dilation reflects effortful action invigoration in overcoming aversive Pavlovian biases

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Abstract

"Pavlovian" or "motivational" biases describe the phenomenon that the valence of prospective outcomes modulates action invigoration: Reward prospect invigorates action, while punishment prospect suppresses it. The adaptive role of these biases in action selection is still unclear. One idea is that these biases constitute a fast-and-frugal decision strategy in situations characterized by novelty, surprise, and threat, e.g., in presence of a predator, which demand a quick response. In this pre-registered study (N = 35), we tested whether such a threatening situation—induced via subliminally presented angry vs. neutral faces—lead to increased reliance on Pavlovian biases. Also, we measured trial-by-trial arousal by tracking pupil diameter while participants performed an orthogonalized Motivational Go/NoGo Task. Pavlovian biases were present in responses, reaction times, and even gaze, with lower gaze dispersion under aversive cues, indicative of "freezing of gaze". The subliminally presented faces did not affect responses, nor reaction times, nor pupil diameter, questioning the effectiveness of this manipulation. However, pupil dilations encoded the task demands, with stronger dilations for Go responses particularly for aversive cues, potentially reflecting the process of learning to recruit effort to overcome aversive inhibition. Taken together, these results point at pupil diameter reflecting effortful action invigoration to overcome freezing induced by aversive cues—a facet of cognitive control unique to the employed task. We discuss our results in the context of noradrenaline and effort expenditure, but also in light of the "value of work" theory of striatal dopamine and the role of basal ganglia pathways in invigorating and suppressing movements.

Key words: motivational biases, Pavlovian, pupillometry, eye-tracker, effort, invigoration

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Humans and other animals are assumed to have different, parallel decision-making systems at their disposal that solve decision problems in different ways (Kahneman, 2011; Loewenstein & O'Donoghue, 2004; Metcalfe & Mischel, 1999; Milli, Lieder, & Griffiths, 2021; Shiffrin & Schneider, 1977). Some of these systems prioritize speed on behalf of accuracy, yielding quick, but seemingly inaccurate or "irrational" decisions. Other systems prioritize accuracy and yield more "rational" decisions, but at the cost of lower speed and increased mental resource demand (Dayan, 2014). One particularly simple, but quick system might be the so-called "Pavlovian" system, responsible for "Pavlovian" or "motivational" biases in behavior (Dayan, Niv, Seymour, & Daw, 2006; Guitart-Masip, Duzel, Dolan, & Dayan, 2014). This system allows the value of cues in the environment—associated with rewards (positive value) or punishments (negative value)—to influence response selection: in the presence of reward-associated stimuli, it invigorates behavior and drives more and faster responses, while in the presence of punishment-associated stimuli, it suppresses behavior and leads to less and slower responses. Given that these biases seem to be altered in depression (Huys et al., 2016; Nord, Lawson, Huys, Pilling, & Roiser, 2018), traumas (Ousdal et al., 2018), anxiety disorders (Mkrtchian, Aylward, Dayan, Roiser, & Robinson, 2017), and alcohol addiction (Chen et al., 2022; Schad et al., 2020), understanding their role in everyday life could shed light on the etiology and maintenance of such motivational disorders.

The presence of multiple decision systems necessitates an arbitration of which system to rely on in a particular situation, potentially driven by which class of situations or ecological niche each system is most "adaptive" in. Previous frameworks have suggested that different decision systems are selected based on their performance in achieving an optimal tradeoff between speed and accuracy (Daw, Niv, & Dayan, 2005; Keramati, Dezfouli, & Piray, 2011; Milli et al., 2021). Under this framework, Pavlovian biases have been suggested to constitute "default response options" in unfamiliar and/ or seemingly uncontrollable environments in which the recruitment of more effortful, "instrumental" control systems does not increase the rate of returned rewards (Dorfman & Gershman, 2019), In such situations, Pavlovian biases might constitute sensible "priors" about which action-outcome contingencies might hold in an environment (Moutoussis et al., 2018). Other frameworks have

characterized Pavlovian control as an "emergency break" that takes over behavior in presence of particularly large rewards or threats, e.g., when facing a dangerous predator (O'Doherty, Cockburn, & Pauli, 2017). Under such circumstances, the Pavlovian system might trump other systems and induce a global inhibition of all motor effectors, characteristic of the freezing response (Roelofs, 2017; Roelofs & Dayan, 2022; Rösler & Gamer, 2019) and commonly induced by unexpected and surprising events (Schmidt & Berke, 2017; Wessel, 2018; Wessel & Aron, 2017). Notably, freezing seems to occur automatically and outside voluntary control, corroborating its likely "Pavlovian" nature.

While a large body of previous research has investigated how imminent threats or unexpected events impact decision-making and induce a bodily stress response, little is known about the role of the Pavlovian system in this response. Under stress, humans show less prospective planning and goal-directedness (Otto, Raio, Chiang, Phelps, & Daw, 2013; Schwabe & Wolf, 2011), less reliance on expected value (Klaassen et al., 2021), and overexploitation of known rather than exploration of new resources (Lenow, Constantino, Daw, & Phelps, 2017). Stressful events induce an acute sympathetic response orchestrated by the neurotransmitter noradrenaline (Bouret & Sara, 2005; Mather, Clewett, Sakaki, & Harley, 2016; Sara, 2009). Tonic stress induction seems to exacerbate both sign-tracking (Anselme, Robinson, & Berridge, 2013) and freezing (Mkrtchian, Roiser, & Robinson, 2017), behaviors taken to reflect Pavlovian biases. While this research suggests that noradrenaline might regulate the recruitment of Pavlovian biases, its direct measurement is challenging in humans. A proxy measure is pupil size, which has been found correlated to activity in the locus coeruleus, the major neural source of noradrenaline (Joshi & Gold, 2019; Strauch, Wang, Einhäuser, Van der Stigchel, & Naber, 2022).

Beyond tonic stress inductions that impact behavior over several minutes, also more subtle, covert cues that induce local, fast fluctuations in arousal have been found to alter decision-making. Specifically, a study using subliminally presented disgusted faces found these cues to induce arousal—as measured by pupil diameter and heart rate acceleration—and exacerbate biases in a perceptual decision-making task (Allen et al., 2016). Another study found supraliminally presented angry faces to induce freezing (Ly, Huys, Stins, Roelofs, & Cools, 2014). Studies measuring instead of manipulating arousal have found associations between pupil diameter (as a proxy for arousal and noradrenaline

levels) and perceptual biases (de Gee et al., 2017; de Gee, Knapen, & Donner, 2014; Urai, Braun, & Donner, 2017), corroborating a link between trial-by-trial fluctuations in arousal and decision strategies. Theoretical frameworks have suggested that phasic noradrenaline encodes an estimate of the unexpected uncertainty in the environment (Aston-Jones & Cohen, 2005; Dayan & Yu, 2006; O'Reilly, 2013; Yu & Dayan, 2005), empirically corroborated by correlations between pupil diameter and uncertainty estimates (Lavín, San Martín, & Rosales Jubal, 2014; Nassar et al., 2012; O'Reilly et al., 2013; Preuschoff, 't Hart, & Einhäuser, 2011). When uncertainty suddenly increases, e.g., an agent observes unexpected outcomes of their actions, it is adaptive for them to forget about action-outcome contingencies learned in the past, which have likely undergone changes, and instead start learning afresh (Bouret & Sara, 2005; Courville, Daw, & Touretzky, 2006; Piray & Daw, 2021; Sara, 2009). In such situations, increased reliance on Pavlovian biases could be adaptive (Dorfman & Gershman, 2019), assuming that these biases constitute plausible "priors" on which action-outcome contingencies might hold in a new environment. In sum, there is reason to assume that high arousal, signaling a state of high uncertainty (potentially indicative of changes in the environment) to the brain, might induce an increased reliance on Pavlovian biases.

To test this idea, we combined the orthogonalized Motivational Go/NoGo Task, a task measuring Pavlovian biases in humans, with a subliminal arousal induction while measuring instantaneous arousal via participants' pupil diameter. We expected that subliminally presented angry (compared to neutral) faces would induce heightened arousal, which should be reflected in stronger pupil dilation. Such arousal should then exacerbate Pavlovian biases. We thus expected an interaction effect between cue valence and arousal on responses, with a stronger valence effect, i.e., more Go response to Win than Avoid cues, in states of high induced and/ or measured arousal.

Methods

Participants and Exclusion Criteria

Sample size (M_{age} = 22.37, SD_{age} = 2.68, range 18–30; 18 women, 17 men; 27 right-handed, 8 left-handed; 18 with right eye dominant; 17 with left eye dominant). The study design, hypotheses, and analysis plan was pre-registered on OSF under https://osf.io/ue397. English-speaking participants in the

age range of 18–35 years old were recruited via the SONA Radboud Research Participation System of Radboud University. Only participants with unimpaired vision or contact lenses were admitted. Exclusion criteria comprised previous neurological treatment, cerebral concussion, brain surgery, or epilepsy. Participants were excluded from all analyses for three (pre-registered) reasons: (a) guessing the hypothesis in the debriefing, (b) performance not significantly above chance (tested by using required action to predict performed action with a logistic regression; only participants with $p < .05$ were maintained); and (c) no pupil data on more than 128 trials (50% of trials). None of these criteria applied to any of the participants. Hence, the final sample size for all analyses comprised $N = 35$. This reported research was approved by the local ethics committee of the Faculty of Social Sciences at Radboud University (proposal no. ECSW-2018-171 and ECSW-2019-055) in accordance with the Declaration of Helsinki.

The sample size was not based on a power analysis, but on lab availability for this project (four weeks, April 16 till May 17, 2019) as this study was conducted as around several thesis projects. The sample size of $N = 35$ was comparable to previous studies investigating Pavlovian biases with the same task (Algermissen, Swart, Scheeringa, Cools, & den Ouden, 2022; Swart et al., 2018) and slightly larger than the study which inspired the subliminal arousal priming manipulation (Allen et al., 2016). A post-hoc sensitivity power analysis yielded that, given 35 participants providing 256 trials (thus 8,960 trials in total), and assuming intra-cluster coefficients of 0.04 for responses, 0.14 for RTs, and 0.17 for dilations (all estimated from the data), the effective sample size was $n = 4{,}090$ for responses, $n = 1{,}558$ for RTs, and $n = 1{,}329$ for dilations, respectively, which allows to detect effects of $\beta > 0.04$ for responses, $\beta > 0.07$ for RTs, and $\beta > 0.08$ for dilations (standardized regression coefficients) with 80% power (Aarts, Verhage, Veenvliet, Dolan, & van der Sluis, 2014).

Procedure

Participants completed a single experimental session that lasted about 45 minutes. They provided informed consent, underwent an 9-point eye-tracker calibration, read computerized instructions and performed four practice trials for each of the four cue conditions. Afterwards, they completed 256 trials of the Motivational Go/NoGo Task. After the task, participants completed

measures of trait anxiety (STAI, Form Y-2, 20 items) (Spielberger, Gorssuch, Lushene, Vagg, & Jacobs, 1983) and impulsivity (UPPS-P short version, five sub scales, 20 items) (Cyders, Littlefield, Coffey, & Karyadi, 2014), which were part of final year theses written on this data set. At the end, participants went through a funnel debriefing asking them what they thought the hypothesis investigated in the experiment was, if they used any strategies not contained in the task instructions (and, if yes, describe them), whether they noticed anything special about the task not mentioned in the instructions (and, if yes, describe it), if they noticed anything special about the face at the beginning of each trial (and, if yes, describe it), whether they recognized the emotions of the face presented very briefly (and, if yes, describe them), and finally, given that there was an angry and a neutral face presented, what they thought the hypothesis investigated in the experiment was. After the completion of the experiment, participants received course credit in compensation plus a performance-dependent candy bar for task accuracy > 75%.

Apparatus

Reporting follows recently suggested guidelines for eye-tracking studies (Fiedler, Schulte-Mecklenbeck, Renkewitz, & Orquin, 2020). The experiment was performed in a dimly lit, sound-attenuated room, with participants' head stabilized with a chin rest. The experimental task was coded in PsychoPy 1.90.3 on Python 2.7, presented on a BenQ XL2420Z screen (1920 x 1080 pixels resolution, refresh rate 144 Hz). People's dominant eye was recorded with an EyeLink 1000 tracker (SR Research, Mississauga, Ontario, Canada; sampling rate of 1,000 Hz; spatial resolution of 0.01° of visual angle, monocular recording). The chinrest was placed about 90 cm in front of the screen and 70 cm in front of the eye-tracker. Before the task, participants underwent the standard 9-point calibration and validation procedure provided by SR Research, which was repeated until error for all nine points was below 1°. The screen background during the task was of the same gray (RGB [166, 166, 166]) as during the calibration. Participants were instructed to focus on the fixation cross/ center of the screen throughout the task. Manual responses (Go) were performed via the space bar of the keyboard.

Task

Participants performed 256 trials (split in four blocks of 64 trials each) of an orthogonalized Motivational Go/ NoGo learning task (Swart et al., 2018). The trial time line was slowed down to reliably measure pupil fluctuations. Each trial started with a series of rapidly presented images used to subliminally induce arousal, followed by a cue indicating the required response and potential outcome of the trial, and finished with the outcome.

The arousal priming manipulation closely followed a procedure previously found effective (Allen et al., 2016). It consisted of a "prime" image presented for 16 ms (two frames), which was either an angry face (image ID AM29ANS; high arousal) or a neutral face (ID AM29NES; low arousal) from the Karolinska face data set (Lundqvist, Flykt, & Öhman, 1998). Hair and background were removed from the face stimulus by cropping it to an elliptical shape (size 281 x 381 pixels; 5.0° x 6.7° visual angle; Fig. 1A). To prevent conscious recognition of the prime stimulus, it was flanked by a forward mask, which was a version of the neutral prime with pixels randomly permuted, presented for 250 ms before the prime, and a backward mask, which was another neutral face taken from the same face data set (ID AM10NES), presented for 100 ms after the prime (Allen et al., 2016). Participants were instructed that the presentation of the backward mask served to keep their attention focused on the task.

Next, participants saw one of four cues for 1,300 ms. During cue presentation, they could make a Go or NoGo response. Participants had to learn from experience whether a cue offered the chance to win points for correct responses (and no change in points for incorrect responses; "Win" cues) or the chance to lose points for incorrect responses (and no change in points for correct responses; "Avoid" cues; Fig. 1C). Also, they needed to learn from trial-and-error whether the cue required a Go response (space bar press) or NoGo response (no press). Cues were of size 300 x 300 pixels (5.3° x 5.3°), presented centrally, set to grayscale and matched for average luminance and local statistical properties using the SHINE toolbox (Willenbockel et al., 2010). Cue assignment to task conditions was counterbalanced across participants. Each cue was presented 16 times in total (eight times with the high arousal and eight times with the low arousal prime), with cue presentation interleaved in a pseudo-randomized way (not more than one consecutive cue repetition). Each of the four blocks featured a new

set of four cues to prevent ceiling effects in performance and to maximize the time during which participants were (at least partially) unsure about the correct response.

After a variable inter-stimulus interval (uniform distribution between 1,300–1,700 ms in steps of 100 ms), the outcome was presented for 700 ms. Outcomes consisted in either money falling into a can (positive feedback for Win cues), money falling out of a can (negative feedback for Avoid cues), or simply a can (negative feedback for Win cues/ positive feedback for Avoid cues). Feedback validity was 75%, i.e., correct responses were followed by positive feedback and incorrect responses followed by negative feedback on 75% of trials, with the reverse being the case on the remaining 25% of trials (Fig. 1C). Trials finished with a variable inter-trial interval (uniform distribution between 2,300–2,700ms in steps of 100 ms).

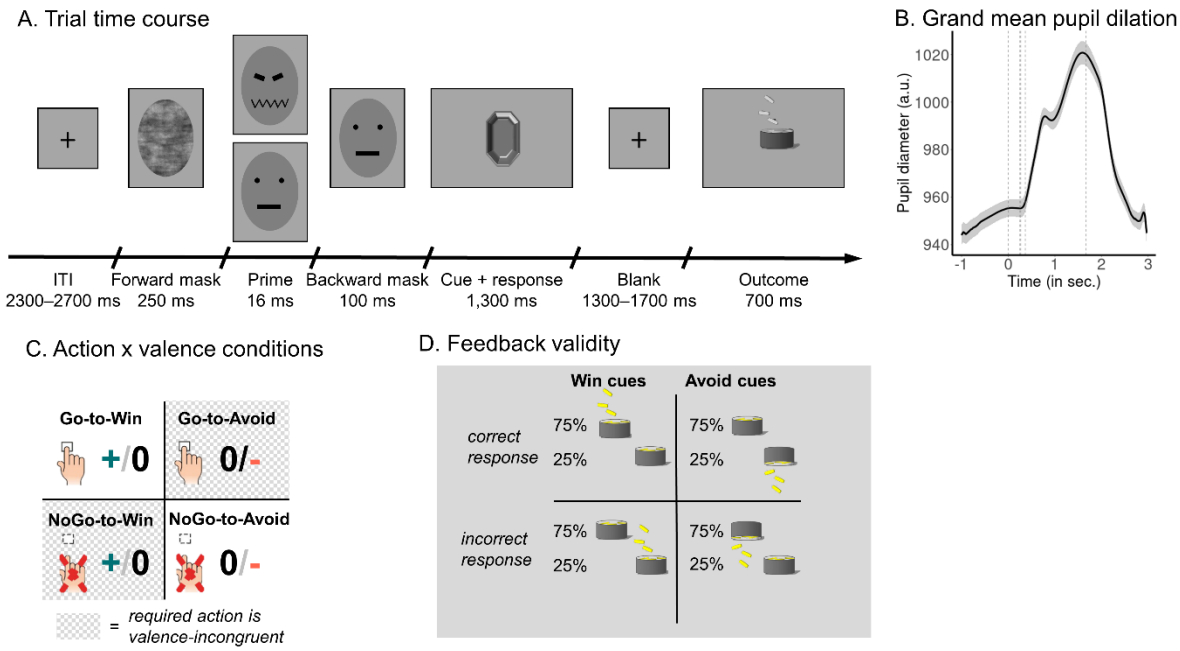

Figure 1. Task design. **A**. *Trial time course*. Each trial starts with a forward mask presented for 250 ms (pixel-permuted version of the neutral prime), a prime stimulus (angry or neutral face; original stimuli replaced to comply with bioRxiv policies) for 16 ms, and a backwards mask (another neutral face) for 100 ms. Participants then see one of four cues and have to decide whether to respond with a button press ("Go") or not ("NoGo"). After a variable interval, the outcome (gain, neutral, loss of points) is shown. **B.** *Grand mean average of the pupil dilation for all trials of all participants*. Vertical dashed lines indicate the onset of the forward mask (at 0 ms), the prime (at 250 ms), the backwards mask (at 266 ms), the cue onset (at 366 ms), and the cue offset (at 1666 ms). **C.** *Task conditions*. Half of the cues are "Win" cues for which participants can gain points, while the other half are "Avoid" cues for which participants can lose points. Orthogonal to the cue valence, one half of the cues requires a Go response ("Go" cues) while the other half requires a NoGo response ("NoGo" cues). **D.** *Feedback given cue valence and accura*cy. For half of the cues ("Win cues"), participants receive mostly gains in points (money falling into a can) for correct responses, but no change in point score (a can) for incorrect responses. For the other half ("Avoid" cues), they receive no change in point score (a can) for correct responses, but a loss of points (money falling out of a can) for incorrect responses.

Data Preprocessing

Behavior

For analyses using RTs, we excluded trials with RTs < 300 ms (in total 36 trials out of 8,960 trials; per participant: M = 1.01, SD = 3.06, range 0–14) since it is implausible that these very fast responses incorporated knowledge about the cue. Note that this step was not pre-registered, but the same procedure was used in previous studies in which we used the same task (Algermissen et al., 2022; Swart et al., 2017). Analyses including all RTs lead to identical conclusions.

Pupil preprocessing

Pupil data were preprocessed in R following previously published pipelines (de Gee et al., 2017; Urai et al., 2017). First, pupil data was epoched into trials from 1,000 ms before until 2,966 ms after forward mask onset (i.e., until the earliest possible end of the ISI/ before possible outcome onset). Note that the pre-registration specifies a different time range (1,000 ms before until 1,666 ms after forward mask onset; i.e. exactly until task cue offset) under the assumption of a peak of the pupil response around 1,000 ms (Hoeks & Levelt, 1993). However, in fact, the grand average pupil response in this data peaked at 1,584 ms (Fig. 1B), i.e., close to the end of the pre-registered time window, with per-trial dilations peaking outside the pre-registered window on almost half of the trials (assuming symmetric noise on the peak latency). The grand average pupil time course only returned to baseline levels around 3,000 ms after forward mask onset (Fig. 1B). We thus decided to extend the time window until 2,966 ms, i.e., until the earliest possible onset of an outcome (Fig. 1A). After epoching, the timing of blinks and saccades (as automatically detected by the EyeLink software) was extracted. These gaps of missing data were zero-padded by deleting 150 ms (for blinks, 20 ms for saccades) of samples before and after them (as recommended by the EyeLink manufacturer). In addition, we computed the first derivative of the pupil time course and marked abnormally fast pupil changes (absolute values of the z-standardized first derivative higher than 2). If two such marks occurred less than 10 samples away from each other, we deleted all samples in-between. Finally, we interpolated missing or deleted samples with linear interpolation and low-pass filtered the data at 6 Hz with a 3-order Butterworth filter. We deleted the first and last 250 ms of each trial to remove edge artifacts caused by the filter. We converted pupil

diameter to units of modulation (percent signal change) around the mean of the pupil time series of each block using the grand-mean pupil diameter per block (i.e., 64 trials forming one block). Trials with more than 50% of missing/ interpolated data were excluded (in total 166 trials out of 8,960 trials; per participant: $M = 4.74$, $SD = 9.10$, range 0–43). Finally, we computed the trial-by-trial pupil baseline as mean pupil diameter in the 500 ms before the onset of the forward mask and the maximal pupil dilation as the maximal value during the 2,966 ms after onset of the forward mask (i.e. until offset of the task cue). We then computed the trial-by-trial pupil dilation by subtracting the trial-by-trial pupil baseline from the trial-by-trial maximal dilation.

Gaze preprocessing

We analyzed the gaze data similar to the pupil data. After epoching, the timing of blinks and saccades (as automatically detected by the Eyelink software) was extracted. These gaps of missing data were zero-padded by deleting 150 ms (for blinks, 20 ms for saccades) of samples before and after them (as recommended by the Eyelink manufacturer). In addition, we computed the first derivative of the pupil time course and marked abnormally fast pupil changes (absolute values of the z-standardized first derivative higher than 2). If two such marks occurred less than 10 samples away from each other, we deleted all samples in-between. We did not apply interpolation for missing gaze data.

Data Analysis

Mixed-effects regression models

For regression analyses, we used mixed-effects linear regression (function lmer) and logistic regression (function glmer) as implemented in the package lme4 in R (Bates, Mächler, Bolker, & Walker, 2015). We used generalized linear models with a binomial link function (i.e., logistic regression) for binary dependent variables (Go vs. NoGo responses) and linear models for continuous variables such as RTs, pupil baseline, and pupil dilation. We used zero-sum coding for categorical independent variables. All continuous dependent and independent variables were standardized such that regression weights can be interpreted as standardized regression coefficients. All regression models contained a fixed intercept. We added all possible random intercepts, slopes, and correlations to achieve a maximal random effects structure (Barr, Levy, Scheepers, & Tily, 2013). *P*-values were computed

using likelihood ratio tests with the package afex (Singmann, Bolker, Westfall, & Aust, 2018). We considered *p*-values smaller than $\alpha = 0.05$ as statistically significant.

As confirmatory models, we fit a mixed-effects logistic regression model with responses (Go/NoGo) as dependent variable and required action (Go/ NoGo), cue valence (Win/ Avoid), arousal priming manipulation (angry/ neutral face), and task-evoked pupil dilations, as well as all possible interactions between them and independent variables. Furthermore, we checked whether including RTs and the interaction between RTs and dilations as covariates of no interest changed results, which was not the case. In case of interactions between dilations and task conditions, in order to better understand these effects, we combined required action and cue valence into a single "cue condition" variable and fit a model with dilation, cue condition, and their interaction. We then tested for differences between conditions in the slope of the dilation effect using *z*-tests provided by the *emmeans* package in R, which corrects for multiple comparisons using the Tukey method.

Cluster-based permutation tests on pupil data

In order to test whether the millisecond-by-millisecond pupil time course during a trial differed between conditions, we used cluster-based permutation tests (Maris & Oostenveld, 2007). We epoched the pre-processed data into trials from -1,000 ms before until 2,966 ms after mask onset, sorted trials into task conditions, and computed the average time course per condition per participant. We then computed a permutation null distribution by, for 10,000 iterations, randomly exchanging the labels of conditions, computing the mean difference between conditions per participant, computing the overall mean difference between conditions across participants, thresholding this difference at $|t| > 2$, computing the sum of *t*-values for each cluster of adjacent samples above threshold (cluster mass), and retaining the largest cluster mass detected for each iteration. We then compared the empirical cluster mass obtained from the actual data to the permutation null distribution and computed the permutation *p*-value as the number of iterations with a larger cluster mass than the empirical cluster mass divided by the total number of iterations. For to correct for pre-trial baseline differences, for each condition, we subtracted the value at time point 0 (also for each iteration in the permutation distribution).

Cluster-based permutation tests on gaze data

In line with previous studies reporting freezing of gaze (Rösler & Gamer, 2019), we used the mean gaze position (x- and y-coordinates) in the 500 ms before mask onset (while the fixation cross from the inter-trial interval was on the screen) as a trial-by-trial baseline and compute the absolute deviation (Euclidean distance in pixels) from that baseline for each trial ("gaze dispersion"). This procedure corrects for any drift in the eye-tracking calibration over time. We then computed the mean distance from the pre-trial baseline at any time point during cue presentation separately for Win and Avoid cues for every participant. We performed a cluster-based permutation test with 10,000 iterations and a cluster-forming threshold of $|t| > 2$ to test for any difference in the distance from the center between Win and Avoid cues.

Generalized additive mixed-effects models

Additive models use smooth functions of a set of predictors (i.e., thin plate regression splines) in order to model a time series. They allow for testing whether the modeled time series differs between conditions. The shape of a smooth function is fitted to the data and can be linear or non-linear, allowing more flexibility in capturing non-linear trends over time compared to linear models, which makes them particularly suited for analyzing pupillometry data (Algermissen, Bijleveld, Jostmann, & Holland, 2019; Baayen, Vasishth, Kliegl, & Bates, 2017; van Rij, Hendriks, van Rijn, Baayen, & Wood, 2019). A smooth function regularizes the raw time courses and in this way suppresses high-frequency (trial-by-trial) noise. It also accounts for non-zero auto-correlation between residuals, which is assumed to be zero in linear models.

In order to test whether the effect of task conditions changed over time, we fit generalized additive mixed-effects models with the trial-by-trial pupil dilation as dependent variable and separate effects of cue repetition (1–16) for each response condition (Go-to-Win, Go-to-Avoid, NoGo-to-Win, NoGo-to-Avoid) as independent variables. We modeled the time course of cue repetition as a factor smooth (which has a similar, but potentially non-linear effect as adding a random intercept and a random slope) for each participant for each block, allowing for the possibility that condition differences were different in different participants in different blocks (equivalent to a full random-effects structure). We used a scaled *t*-distribution instead of a Gaussian distribution for the response variable in case it led to

lower fREML values (which was the case for both pupil baselines and dilations). In case of significant residual auto-correlation at lag 1 (which was the case for baselines), we added an AR(1) auto-regressive model, with the proportionality constant set to the lag 1-correlation of the residuals from the model without the AR(1). For all fitted models, we visually checked that residuals were approximately normally distributed using quantile-quantile plots and whether auto-correlation was near zero using auto-correlation plots (van Rij et al., 2019).

Transparency and openness

We report how we determined our sample size, all data exclusions, all manipulations, and all measures in the study. All data, analysis code, and research materials will be shared upon publication. The study design, hypotheses, and confirmatory analysis plan were pre-registered under https://osf.io/57zjh and updated under https://osf.io/azqjt (extending data collection by one week). We deviated from the pre-registration in the definition of the time window in which pupil dilation was defined. The pre-registration also specified plans for computational models and a deconvolution GLM approach. We did not pursue the computational modelling because we did not observe any effect of the arousal priming manipulation or the trial-by-trial pupil dilation on the size of Pavlovian biases. Similarly, given that the deconvolution GLM would only have replicated previous findings about outcome-related pupil dilation, but not tested the impact of the manipulation or pupil dilation on Pavlovian biases, it was eventually not pursued. Data were analyzed using R, version 4.1.3 (R Core Team, 2022). Models were fitted with the package lme4, version 1.1.31 (Bates et al., 2015). Plots were generated with ggplot, version 3.4.2 (Wickham, 2016).

Results

Manipulation checks: Learning and Pavlovian bias

First, in line with the pre-registration, we performed manipulation checks to replicate effects typically found with this task (Algermissen et al., 2022; Swart et al., 2018). We fit a mixed-effects logistic regression with responses (Go/ NoGo) as dependent variable and required action (Go/ NoGo) and cue valence (Win/ Avoid) as well as their interaction as independent variables (see Supplementary Material S01 for an overview of all regression results; see Supplementary Material S02 for means and

standard deviations per condition). Participants performed significantly more (correct) Go responses to Go cues than (incorrect) Go responses to NoGo cues (required action), b = 1.367, 95%-CI [1.178, 1.556], $\chi^2(1) = 66.523$, $p < .001$, indicating that participants successfully learned the task (Fig. 2A-C). Also, they performed more Go responses to Win than to Avoid cues (cue valence), b = 0.538, 95%-CI [0.341, 0.734], $\chi^2(1) = 20.986$, $p < .001$, reflecting the Pavlovian bias (Fig. 2A-C). The interaction between required action and valence was not significant, b = 0.068, 95%-CI [-0.044, 0.181], $\chi^2(1) = 1.348$, $p = .246$, providing no evidence that the Pavlovian bias was stronger for Go or for NoGo cues.

Furthermore, we fit a mixed-effects linear regression with RTs as dependent variable and again required action, cue valence, and their interaction as independent variables. This analysis was omitted in the pre-registration, but in line with previous studies (Algermissen et al., 2022). Participant were faster at (correct) Go responses to Go cues than (incorrect) Go responses to NoGo cues (required action), b = -0.143, 95%-CI [-0.197, -0.088], $\chi^2(1) = 20.446$, $p < .001$ (Fig. 2D-F). Also, they were faster at performing responses to Win than to Avoid cues (cue valence), b = -0.143, 95%-CI [-0.197, -0.088], $\chi^2(1) = 27.329$, $p < .001$, again reflecting the Pavlovian biases (Fig. 2D-F). The interaction between required action and valence was not significant, b = -0.007, 95%-CI [-0.051, 0.037], $\chi^2(1) = 0.083$, $p = .773$, providing no evidence that the Pavlovian bias was stronger for Go or for NoGo cues. Pavlovian biases for neither responses nor RTs were correlated with trait anxiety or impulsivity across participants (Supplementary Material S03). Taken together, these results corroborate that participants learned the task and exhibited Pavlovian biases.

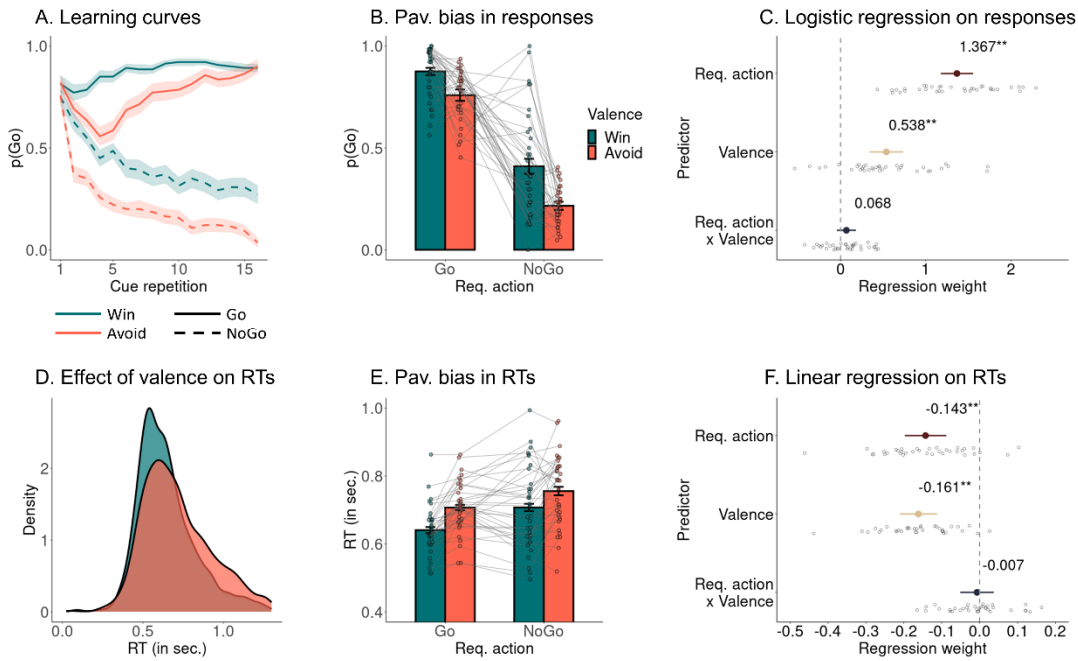

Figure 2. *Effect of required action and cue valence on responses and RTs*. **A.** Trial-by-trial proportion of Go responses per cue condition. Participants learn to perform a Go response or not, with significantly more Go responses to Go cues than NoGo cues. Also, they perform significantly more Go responses to Win cues than to Avoid cues, reflecting the Pavlovian bias. Note that participants are initially unaware of the cue valence and have to infer it from (non-neutral) feedback, which explains why the bias only emerges after the first few trials. For the Go-to-Avoid conditions, the bias initially suppresses responding, and participants have to subsequently learn to overcome the bias and perform a Go response. This is reflected in the dip in Go responses for Go-to-Avoid cues for trials 1–5 when the negative valence of this cue is learned, and a subsequent rise in Go responding as the correct response to this cue is learned. Error bands are ± SEM across participants. **B.** Proportion of Go responses per cue condition (whiskers are ± SEM across participants, dots indicate individual participants). Participants show significantly more Go responses to Go than NoGo cues (reflecting learning) and significantly more Go responses to Win cues than Avoid cues (indicative of Pavlovian biases). **C.** Group-level (colored dot, 95%-CI) and individual-participant (grey dots) regression coefficients from a mixed-effects logistic regression of responses on required action, cue valence, and their interaction. **D**. Distribution of raw RTs separately per cue valence. **E.** Mean RTs per cue condition. Participants show significantly faster (correct) Go responses to Go than (incorrect) Go responses to NoGo cues and significantly faster responses to Win cues than Avoid cues (indicative of Pavlovian biases). **F.** Group-level and individual-participant regression coefficients from a mixed-effects linear regression of RTs on required action, cue valence, and their interaction.

Exploratory analyses: Freezing of gaze induced by aversive cues

Previous research on humans and animals has investigated the phenomenon of "freezing", i.e., temporarily reduced body motion in presence of a thread (Blanchard, 2017; Roelofs, 2017). Freezing in humans is typically measured via reductions in heart rate (Hashemi et al., 2019; Klaassen et al., 2021) and bodily mobility (Ly et al., 2014) tracked with a stabilometric force-platform that records spontaneous fluctuations in body sway. Recently, it has been suggested that freezing might also affect gaze, with a stronger center bias and less visual exploration while participants prepare a response to avoid an electric shock (Merscher & Gamer, 2024; Merscher, Tovote, Pauli, & Gamer, 2022; Rösler & Gamer, 2019). Here, we tested whether a similar freezing of gaze pattern occurred during the presentation of Avoid compared to Win cues in the context of the Motivational Go/NoGo Task, testing for a difference in the absolute distance from the center of the screen ("gaze dispersion") between trials with Win and Avoid cues.

A cluster-based permutation test in the time range of 0–500 ms after cue onset was significant (p = .024, two-sided; driven by a cluster above threshold 202–278 ms after cue onset; Fig. 3A, B). Distance from the center was lower on trials with Avoid cues than on trials with Win cues, in line with the idea of "freezing of gaze" induced by Avoid cues. Computing the maximal distance from the center in this time window for every trial, averaging distances for Win and Avoid cues per participant, and then averaging across participants confirmed this difference (Fig. 3C). Importantly, there was no difference in gaze dispersion between Win and Avoid cues on the first five repetitions of a cue, i.e., when participants were not fully aware of cue valence yet (Fig. 3D; no cluster above threshold), but this difference only emerged on cue repetitions 6–10 (Fig. 3E; p = .009, cluster above threshold from 242–281 ms after cue onset) and became stronger for cue repetitions 11–15 (Fig. 3F; multiple disconnected clusters above threshold between 55–353 ms after cue onset; largest cluster above threshold from 245–261 ms, p = .023).

In sum, we found evidence for freezing of gaze induced by Avoid cues, with lower gaze dispersion on trials with Avoid cues compared to trials with Win cues. This difference in gaze dispersion only emerged with learning the aversive nature of cues.

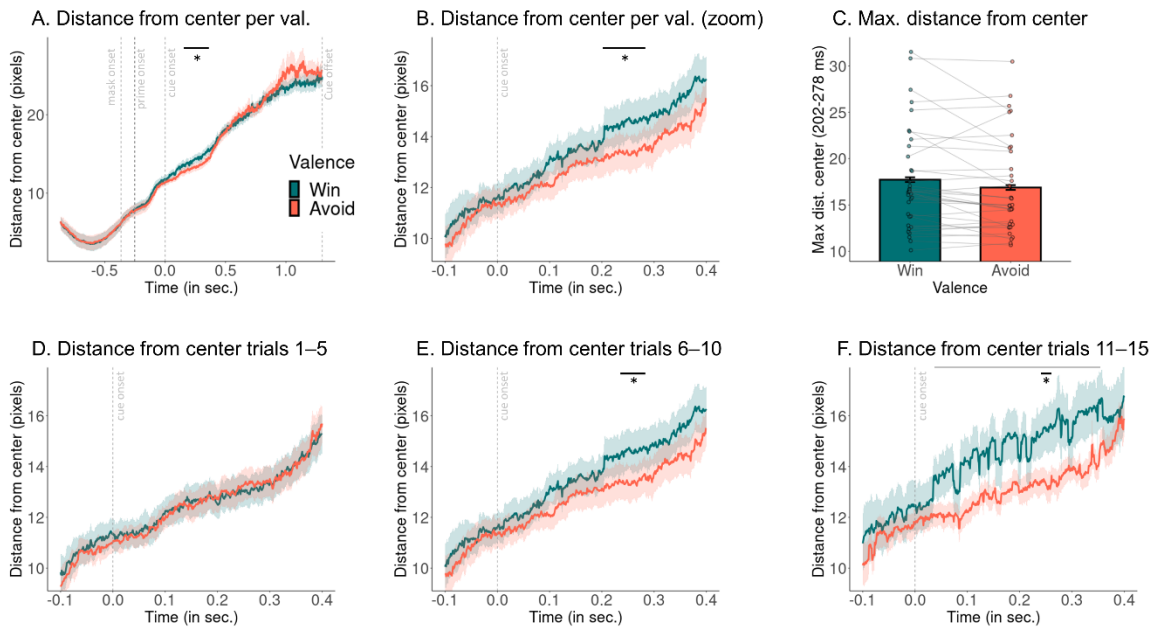

Figure 3. Freezing of gaze induced by Avoid cues. **A.** Mean distance from the gaze position during the trial-by-trial baseline ("center"). Vertical dashed lines indicate the onset of the forward mask (at -366 ms), the prime (at -266 ms), the backwards mask (at -250 ms), the cue onset (at 0 ms), and the cue offset (at 1300 ms). Distance increases with time. Around 202–278 ms after cue onset, distance from the center is lower on trials with Avoid cues compared to trials with Win cues. **B**. Same as panel A, but zoomed into the time range of -100–400 ms after cue onset. **C**. Maximum distance from the pre-trial baseline (whiskers are ± SEM across participants, dots indicate individual participants) averaged for Win and Avoid cues for each participant. Distance is lower on trials with Avoid cues compared to trials with Win cues. **D-F.** Same as panel B, but computed for subsets of trials. While freezing of gaze is absent on the first five cue repetitions when participants are not yet fully aware of the cue valence (see learning curves in Fig. 2A), with no cluster above threshold (**D**), the freezing of gaze bias emerges on cue repetitions 6–10 (**E**; p = .009; cluster above threshold 242–281 ms after cue onset) and becomes even stronger on cue repetitions 11–15 (**F**; multiple disconnected clusters above threshold between 55–353 ms after cue onset, grey horizontal line; largest cluster above threshold from 245–261 ms, p = .023, black horizontal line).

Confirmatory analyses: No effect of the arousal priming manipulation on responses and RTs

As a first set of confirmatory, pre-registered analyses, we extended the above regression model fitting responses as a function of required action and cue valence by adding the arousal priming manipulation (high/ low, i.e., angry/ neutral face stimulus) and all higher-order interactions possible. Neither the main effect of the arousal priming manipulation, b = -0.008, 95%-CI [-0.063, 0.047], $\chi^2(1)$ = 0.054, p = .816, nor the 2-way interaction between the priming manipulation and cue valence, b = 0.006, 95%-CI [-0.052, 0.065], $\chi^2(1)$ = 0.034, p = .854, nor the 3-way interaction between the priming manipulation, cue valence, and required action, b = -0.014, 95%-CI [-0.071, 0.043], $\chi^2(1)$ = 0.170, p = .680, was significant, providing no evidence for any effect of the priming manipulation on responses (Fig. 4A-C).

Fitting an equivalent model to RTs, neither the main effect of the arousal priming manipulation, b = -0.005, 95%-CI [-0.038, 0.028], $\chi^2(1)$ = 0.073, p = .787, nor the 2-way interaction between the priming manipulation and cue valence, b = 0.008, 95%-CI [-0.026, 0.043], $\chi^2(1)$ = 0.197, p = .657, nor the 3-way interaction between the priming manipulation, cue valence, and required action, b = -0.025, 95%-CI [-0.055, 0.006], $\chi^2(1)$ = 2.354, p = .125, was significant, providing no evidence for any effect of the arousal priming manipulation on responses (Fig. 4D-F). Neither the effect of the arousal priming manipulation on responses nor on RTs was correlated with trait anxiety or impulsivity across participants (Supplementary Material S03). Taken together, none of the confirmatory analyses provided any evidence for the arousal priming manipulation affecting behavior.

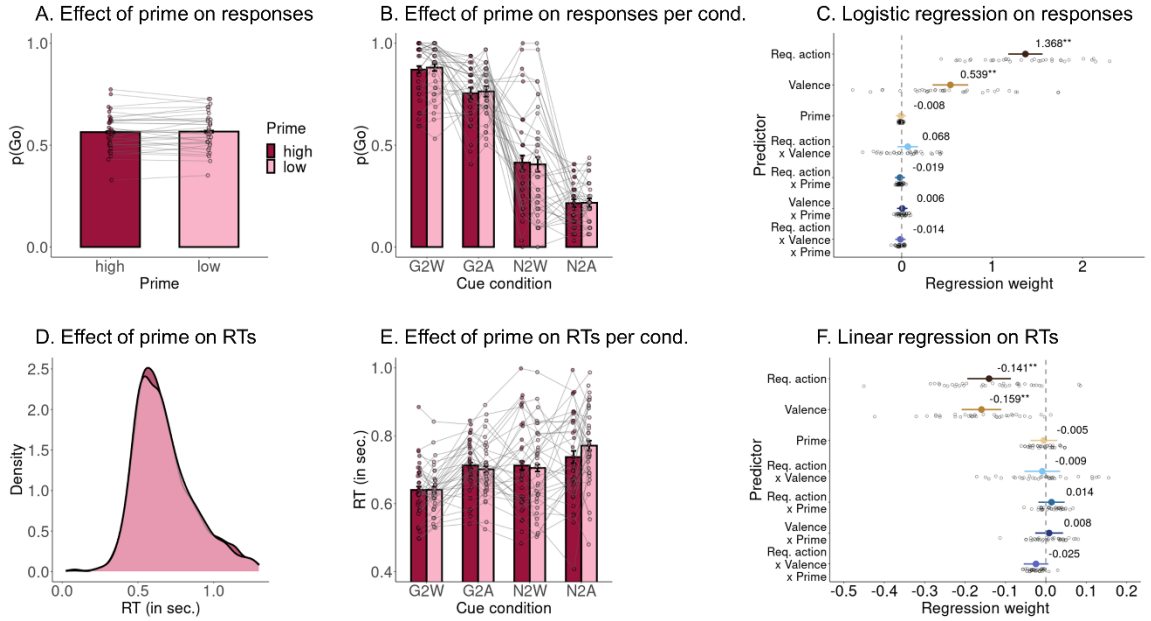

Figure 4. Effect of the arousal priming manipulation on responses and RTs. **A.** Proportion of Go responses for high (angry face) and low arousal (neutral face) priming manipulation (whiskers are ± SEM across participants, dots indicate individual participants). There is no effect of the manipulation on responses. **B**. Proportion of Go responses for high and low arousal priming manipulation separately per cue condition. There is no effect of the manipulation on responses for any condition. **C.** Group-level (colored dot, 95%-CI) and individual-participant (grey dots) regression coefficients from a mixed-effects logistic regression of responses on required action, cue valence, the arousal priming manipulation, and all higher-order interactions. None of the terms involving the arousal priming manipulation is significant. **D**. Distribution of raw RTs separately per arousal priming manipulation level. There is no difference between both levels. **E**. Mean RTs for high and low arousal priming manipulation separately per cue condition. There is no effect of the manipulation on RTs for any condition. **F.** Group-level and individual-participant regression coefficients from a mixed-effects linear regression of RTs on required action, cue valence, the arousal priming manipulation, and all higher-order interactions. None of the terms involving the arousal priming manipulation is significant.

Confirmatory analyses: Association of pupil dilations with responses and RTs

As a second set of confirmatory analyses, we extended the above regression model fitting responses as a function of required action and cue valence by adding the trial-by-trial pupil dilation and all possible higher-order interactions. There was a significant main effect of dilation, b = 0.309, 95%-CI [0.203, 0.414], $\chi^2(1)$ = 22.519, p < .001, with overall stronger dilations for Go responses (Fig. 5A, C), as well as significant interaction between dilations and required action, b = -0.119, 95%-CI [-0.19, -0.049], with a stronger association between Go responses and dilations for NoGo than for Go cues (Fig. 5B, C). In contrast, neither the 2-way interaction between dilations and cue valence b = -0.004, 95%-CI [-0.085, 0.077], $\chi^2(1)$ = 0.009, p = .923, nor the 3-way interaction between dilations, cue valence, and required action was significant, b = -0.012, 95%-CI [-0.095, 0.071], $\chi^2(1)$ = 0.065, p = .799, providing no evidence for pupil dilation modulating the effect of Pavlovian biases on responses (Fig. 5C). To better understand the 2-way interaction between dilations and required action, we fit a

follow-up model combining required action and cue valence into a single "cue condition" variable with 4 levels (Go-to-Win, Go-to-Avoid, NoGo-to-Win, NoGo-to-Avoid). The 2-way interaction between dilations and conditions was significant, $\chi^2(1) = 8.977$, $p = .030$. The association between dilation and the probability of making a Go response was positive in all conditions, with a marginally significant tendency for a stronger link for NoGo-to-Win cues than for Go-to-Win cues ($z = 2.409$, $p = 0.076$) and for Go-to-Avoid cues ($z = 2.406$, $p = .076$), but overall no significant difference between pairs of conditions. See Supplementary Material S04 for evidence that the stronger dilations for incorrect responses (to NoGo cues) than correct responses (to Go cues) occurred due to the former being overall slower, with the association between pupil dilations and accuracy vanishing when controlling for RT differences. In sum, Go responses were associated with stronger pupil dilation, especially for NoGo cues (i.e., when those responses were incorrect and slow), but there was no evidence for dilations modulating the Pavlovian bias in responses.

An equivalent model fit to RTs yielded a significant main effect pupil dilation, $b = 0.096$, 95%-CI [0.062, 0.129], $\chi^2(1) = 43.879$, $p < .001$, with stronger dilations being associated with slower RTs, and a significant 2-way interaction between dilations and required action, $b = 0.039$, 95%-CI [0.007, 0.072], $\chi^2(1) = 5.338$, $p = .021$, with a stronger link between dilations and RTs for Go cues compared to NoGo cues (Fig. 5E, F). The 2-way interaction between dilations and cue valence was only marginally significant, $b = -0.034$, 95%-CI [-0.070, 0.002], $\chi^2(1) = 3.140$, $p = .076$, tending towards a stronger link between dilations and RTs for Avoid compared to Win cues (which would imply a stronger Pavlovian bias under high compared to low dilations; Fig. 5D, F). The 3-way interaction between dilations, cue valence, and required action was no significant, $b = 0.004$, 95%-CI [-0.03, 0.038], $\chi^2(1) = 0.057$, $p = .812$ (Fig. 5F). Neither the effect of the pupil dilation on responses nor on RTs was correlated with trait anxiety or impulsivity across participants (Supplementary Material S03). To better understand the (marginally) significant 2-way interactions, i.e., test explicitly whether effects were driven by only one of the four cue conditions, we again fit a follow-up model combining required action and cue valence into a single "cue condition" variable with four levels. The 2-way interaction between dilation and cue condition was significant, $\chi^2(1) = 9.603$, $p = .023$. The association between dilations

and RTs was positive in all conditions, strongest in the Go-to-Avoid condition, and weakest in the NoGo-to-Win condition, with this difference being significant, $z = 3.339$, $p = .005$, but none of the other comparisons being significant $p > .108$. See Supplementary Material S04 for evidence that the association between strong pupil dilations and slow RTs also explains the association between pupil dilations and incorrect responses. In sum, stronger dilations were associated with slower RTs, especially so for Go cues and Avoid cues, exacerbating the Pavlovian bias in RTs.

As a third set of confirmatory analyses, we fit a regression model with required action, cue valence, the arousal priming manipulation, pupil dilation, and all higher-order interactions possible. There was no significant 4-way interaction on either responses, $b = 0.027$, 95%-CI [-0.044, 0.098], $\chi^2(1) = 0.420$, $p = .517$, nor RTs, b = 0.024, 95%-CI [-0.006, 0.055], $\chi^2(1) = 3.817$, $p = .051$, again providing no evidence for an effect of the arousal priming manipulation, also not as a function of the trial-by-trial pupil dilation.

In sum, pupil dilations were stronger for Go responses, particularly for slow (and incorrect) responses. The link between pupil dilation and RTs was stronger for Avoid cues, perhaps suggesting that strong dilations exacerbate Pavlovian biases in RTs (i.e., lead to a larger difference in RTs between Avoid and Win cues). However, this effect was only marginally significant and appeared to be driven by responses to Go-to-Avoid (rather than NoGo-to-Avoid) cues (though note that, for the latter, Go responses were incorrect, and thus only few trials with RTs were available). Next, we conducted further exploratory, non-preregistered analyses to test whether the arousal priming manipulation had any effect on pupil dilation, and to understand how pupil dilation was modulated by the task factors.

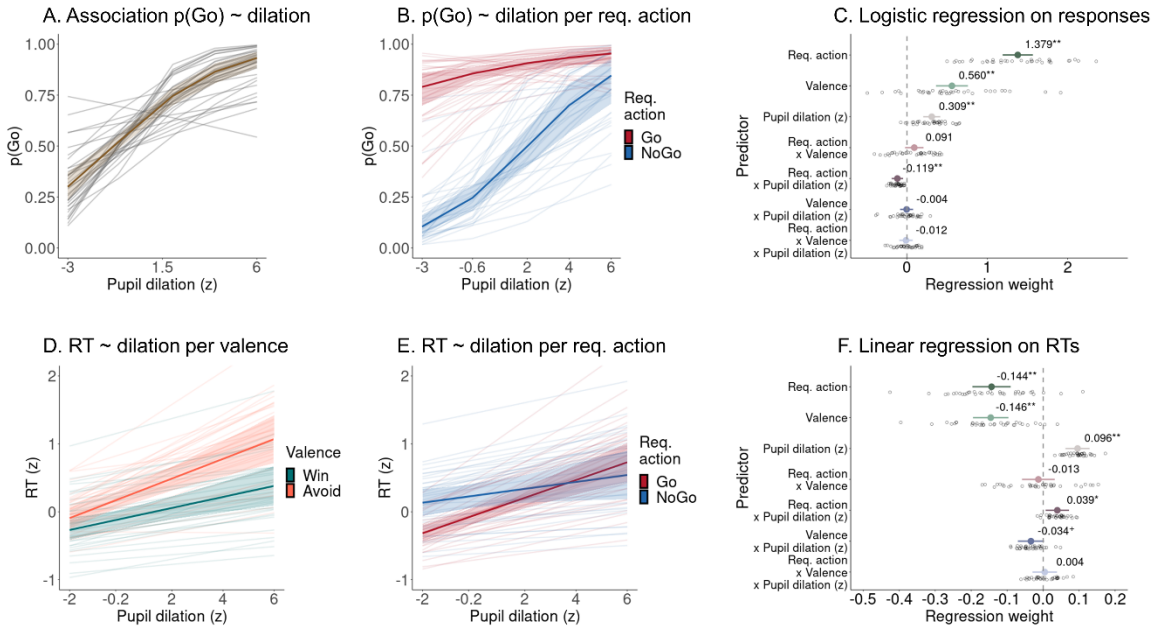

Figure 5. Effect of the trial-by-trial pupil dilation on responses and RTs. **A.** Proportion of Go responses as a function of trial-by-trial pupil dilation as predicted from a mixed-effects logistic regression model (colored line and shades are the group-level association + 95%-CIs; individual lines are the predictions for each individual participant). Go responses are associated with stronger pupil dilations. **B**. Predictions from panel A split per required action. The association between responses and pupil dilations is significantly stronger for (incorrect) responses to NoGo cues than for (correct) responses to Go cues. This difference between incorrect and correct responses is likely due to the former being slower than the latter (see Supplementary Material S04). **C.** Group-level (colored dot, 95%-CI) and individual-participant (grey dots) regression coefficients from a mixed-effects logistic regression of responses on required action, cue valence, pupil dilation, and all higher-order interactions. The main effect of pupil dilation and its interaction with required action are significant. **D**. Predictions of RTs from a mixed-effects logistic regression model based on trial-by-trial pupil dilation separately for Win and Avoid cues. Stronger pupil dilations are associated with slower responses. This relationship is marginally significantly stronger for Avoid than for Win cues. **E**. Predictions of RTs from a mixed-effects logistic regression model based on trial-by-trial pupil dilation separately for Go and NoGo cues. The association between pupil dilation and RTs is significantly stronger for (correct) responses to Go cues than (incorrect) responses to NoGo cues. **F.** Group-level and individual-participant regression coefficients from a mixed-effects linear regression of RTs on required action, cue valence, pupil dilation, and all higher-order interactions. The main effect of pupil dilation as well as its interaction with required action is significant; its interaction with cue valence is marginally significant.

Exploratory analyses: Arousal priming manipulation does not affect pupil dilation

As an additional check of whether the arousal priming manipulation had any effect on cognitive processing, we tested whether it affected pupil dilation. There was no effect of the manipulation on pupil dilation, b = -0.003, 95%-CI [-0.022, 0.017], $\chi^2(1) = 0.071$, p = .790 (Fig. 6A). Also when investigating the raw pupil time course within a trial or the time course of dilations across trials within a block, no difference between the arousal priming manipulations emerged at any time point, and neither was the effect of arousal on dilations correlated with its effect on responses or RTs (see Supplementary Material S05). In sum, the arousal priming manipulation neither affected pupil dilation nor responses nor RTs, suggesting that the manipulation was ineffective and participants did not process the facial emotion. This conclusion aligns with the fact that none of the participants had consciously noticed the

angry or neutral face. See the discussion section for a more general evaluation of the subliminal priming manipulation.

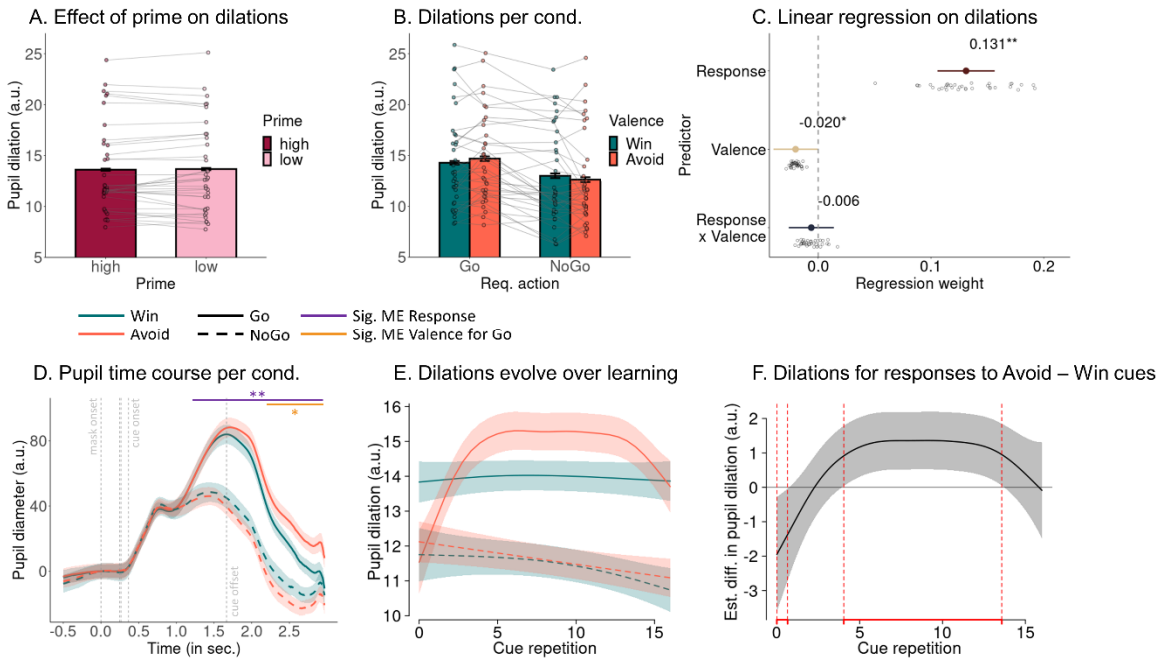

Figure 6. Dilation as a function of the arousal priming manipulation and cue conditions. **A.** Mean pupil dilation per level of the arousal priming manipulation (whiskers are ±SEM across participants, dots indicate individual participants). There is no effect of the arousal priming manipulation on pupil dilations. **B.** Mean pupil dilation per response and cue valence. Dilations are significantly higher for Go than NoGo responses and significantly higher for Go responses to Avoid cues than responses to Win cues. **C.** Group-level (colored dot, 95%-CI) and individual-participant (grey dots) regression coefficients from a mixed-effects linear regression of dilations on response, cue valence, and their interaction. There are significant main effects of response and cue valence, but the interaction is not significant. **D**. Pupil time course within a trial locked to forward mask onset per response per cue valence (mean ± SEM across participants; baseline-corrected). Vertical dashed lines indicate the onset of the forward mask (at 0 ms), the prime (at 250 ms), the backwards mask (at 266 ms), the cue onset (at 366 ms), and the cue offset (at 1666 ms). The pupil dilates significantly more on trials with Go responses than on trials with NoGo responses starting 1,190 ms after forward mask onset (purple horizontal line). Furthermore, the pupil dilates significantly more sustainedly for responses to Avoid than to Win cues, starting 2,157 ms after forward mask onset (orange horizontal line). See Supplementary Material S06 for a version without baseline correction. **E.** Time course of dilations over cue repetitions (mean ± SE) as predicted from a generalized additive mixed-effects model (GAMM), separated by response and cue valence. Dilations are significantly stronger on trials with Go responses than on trials with NoGo responses through blocks. Furthermore, dilations are significantly stronger for responses to Avoid cues than to Win cues from cue repetition 3 to 13, putatively reflecting heightened effort recruitment on trials with Avoid cues in order to overcome aversive inhibition. **F.** Difference line between dilations on trials with responses to Avoid cues minus Win cues. Areas highlighted in red indicate time windows with significant differences.

Exploratory analyses: Stronger trial-by-trial pupil dilations for Go responses, especially to Avoid cues

There was a significant effect of the required action on pupil dilation, $b = 0.078$, 95%-CI [0.057, 0.098], $\chi^2(1) = 34.120$, $p < .001$. However, adding the actually performed response to the model markedly attenuated this effect, $b = 0.026$, 95%-CI [0.003, 0.049], $\chi^2(1) = 4.736$, $p = .030$, with the actual response showing a much stronger effect, $b = 0.112$, 95%-CI [0.084, 0.140], $\chi^2(1) = 38.769$, $p < .001$ (Fig. 6B, C). In line with the above results, strong pupil dilations were present when performing a Go response rather than merely seeing a Go cue that required such a response. Hence, all following

analyses use the performed response instead of the required action as an independent variable. A regression model with dilations as dependent variable and response and cue valence as independent variables yielded, on top of a highly significant main effect of performed response (see above), a significant main effect of valence, b = -0.020, 95%-CI [-0.040, -0.001], $\chi^2(1) = 4.007$, $p = .045$, with stronger dilation for Avoid than Win cues (Fig. 6B, C). The interaction between performed action and valence was not significant, b -0.006, 95%-CI [-0.026, 0.014], $\chi^2(1) = 0.356$, $p = .551$. Note however that the pattern displayed in Fig. 6B is suggestive of an interaction effect, with higher dilations for Avoid than Win cues only for Go responses, with this pattern reversing for NoGo responses. This observation was confirmed when using post-hoc z-tests, which yielded a significant effect of valence only for Go responses, $z = 1.974$, $p = .048$, but not for NoGo responses, $z = 0.915$, $p = .360$. We followed up on this inconsistency between regression results (Fig. 6C) and the pattern observed when plotting the data (Fig. 6B) with further analyses.

In sum, pupil dilation was stronger for Go than NoGo cues and also modulated by cue valence, with stronger dilations to Avoid than Win cues. However, the pattern of significant and non-significant effects in the regression model (Fig. 6C) did not match the per-condition raw data (Fig. 6B). To further explore the dynamics of pupil dilation within each trial, we analyzed the full pupil time course using cluster-based permutation tests.

Exploratory analyses: Effects of task conditions on the pupil time course

The previous analyses focused on the trial-by-trial peak of the pupil time course, which is a frequently used summary statistic of the pupil time course, but does not capture any variation beyond the peak height, such as differences in peak timing between conditions. Given the above-reported inconsistency between regression results and patterns observed when plotting the data, as a more sensitive measure of condition differences, we tested for such differences in the millisecond-by-millisecond pupil time course using cluster-based permutation tests (Strauch et al., 2022). We corrected for any pre-onset baseline differences (for results without baseline correction, see Supplementary Material S06). The pupil was significantly wider on trials with Go compared to trials with NoGo responses, $p < .001$, driven by a cluster above threshold from 1,190–2,966 ms after mask onset (i.e.,

until the end of the testing window, Fig. 6D). Within this timeframe, the pupil was significantly wider for Go responses to Avoid cues than Go responses to Win cues, p = .035, driven by a cluster above threshold from 2,157–2,966 ms (i.e., until the end of the testing window; Fig. 6D). Note that this difference occurred rather late, i.e., after the peak of the grand mean pupil response (at 1,591 ms) and after the task cue had already disappeared (i.e., after 1,666 ms). Despite its late time point, due to the sluggishness of the pupil response, it might reflect differences in cognitive processing occurring much earlier, i.e., during cue processing and response selection. Note that this difference occurred much later than differences in gaze dispersion between Avoid and Win cues (i.e., 202–278 ms after cue onset); freezing of gaze and difference and pupil dilation are thus unlikely to confound each other. Taken together, the pupil time course during Go responses to Avoid cues was only marginally higher at the peak compared to the time course during Go responses to Win cues, but significantly more sustained. There was no significant difference between NoGo responses to Win and to Avoid cues, p = 1, with no cluster above threshold. Without baseline-correction, differences between responses to Avoid and Win cues were in fact substantially larger (see Supplementary Material S06). For associations between task factors and outcome-locked pupil dilations, see Supplementary Material S07.

These results suggest that the main effect of cue valence on trial-by-trial pupil dilation found in the regression models above is more accurately described as a prolonged duration of the Go-associated pupil dilation for Avoid cues. A putative interpretation of this pattern is that Go responses to Avoid cues require increased effort in order to overcome aversive inhibition elicited by Pavlovian biases. Notably, this pattern should only evolve with learning as participants come to realize the cue valence and the response required for a given cue, and it might disappear again once the task is well learned. Hence, next, we tested for condition differences in the dilation time course within task blocks and how they changed with learning.

Exploratory analyses: Effects of task conditions on pupil dilation over time

Analyses of the trial-by-trial dilations as well as of the pupil time courses suggested slightly stronger pupil responses during Go responses to Avoid cues compared to Go responses to Win cues. Specifically, while some research has interpreted pupil dilations to be induced by movement preparation

and execution in an all-or-none fashion (Richer & Beatty, 1985), other research has interpreted pupil dilation to reflect effort recruitment in a more graded fashion (van der Wel & van Steenbergen, 2018). In the context of the Motivational Go/NoGo task, higher effort is likely to be required for Go responses to Avoid cues than to Win cues because aversive inhibition (i.e., the Pavlovian bias) has to be overcome. It can be assumed that participants are initially unaware of the correct response or cue valence and thus do not recruit additional effort to invigorate Go responses to Avoid cues (see learning curve per cue in Fig. 2A). As they become more certain about which response to perform, effort recruitment might increase, particular for the cues they have learned to be Avoid cues. With further learning, response selection becomes more certain and the instrumental system dominates the Pavlovian system, requiring less effort with increasing practice. As a result of these two antagonistic trends, an inverted-U shape, with maximal effort recruitment at intermediate stages of learning, could be expected. To test this hypothesis, we fit generalized additive mixed-effects models to participants' trial-by-trial pupil dilations, testing whether the time course of pupil dilations (modeled via the cue repetition, 1–16) differed between conditions.

The model suggested significantly higher pupil dilations for Go than NoGo responses throughout learning (repetitions 1–16), parametric term $t(5.54, 7.45) = 14.585$, $p < .001$, smooth term $F(1.32, 1.56) = 2.340$, $p = .165$. Furthermore, pupil dilations were significantly stronger for Go responses to Avoid cues than to Win cues between cue repetitions 3 till 13 (and lower around cue repetition 1), parametric term $t(5.75, 7.67) = 3.039$, $p = .002$, smooth term $F(3.39, 4.16) = 3.483$, $p = .007$ (Fig. 6E, F). Note how this time course is mirroring the learning curve for Go-to-Aoid cues (Fig. 2A). See Supplementary Material S04 for results showing that this pattern held independently of other factors affecting pupil dilations for Go responses, such as accuracy, response speed, and response repetition.

In sum, these results indicate that stronger dilations for Go responses to Avoid compared to Win cues occurred specifically at intermediate stages of learning, when overcoming aversive inhibition has become driven by past experiences, but not sufficiently practiced yet.

Discussion

In this study, we tested whether induced or measured arousal indexed via pupil dilation modulated Pavlovian biases in an orthogonalized Motivational Go/NoGo Task. Win vs. Avoid cues induced strong Pavlovian biases in responses, RTs, and even gaze position, with less gaze dispersion from the center for Avoid compared to Win cues, indicative of a "freezing of gaze" induced by aversive cues. Neither responses, nor RTs, nor pupil dilations showed any effect of the arousal priming manipulation, questioning the effectiveness of the manipulation used in this study (Allen et al., 2016). In contrast to the priming manipulation, arousal measured via trial-by-trial pupil dilation reflected task factors: stronger dilations occurred on trials with Go responses, particularly for slow responses and responses to Avoid cues. Lastly, stronger pupil dilations for Go responses to Avoid cues only emerged with learning, indicative that they do not reflect motor processes per se, but the specific effort demands required to push through a Go response in face of aversive inhibition. Beyond previous literature on conflict detection and response suppression in the context of Pavlovian biases (Cavanagh, Eisenberg, Guitart-Masip, Huys, & Frank, 2013; Swart et al., 2018), these results highlight another cognitive capacity required to manage Pavlovian biases, namely response invigoration against adversities, which potentially involves noradrenergic mechanisms.

Freezing of gaze by aversive cues

Aversive cues robustly reduced response rates and slowed reaction times. Note that strong aversive Pavlovian biases are usually absent in variants of the Motivational Go/NoGo Task that separate Pavlovian cues and the response window in time (Guitart-Masip et al., 2012; Queirazza, Steele, Krishnadas, Cavanagh, & Philiastides, 2023). Hence, the instruction to respond immediately to the appearance to the cue seems necessary for observing these biases in behavior. Only in such a variant, it becomes possible to study the mechanisms by which participants overcome an aversive bias.

Beyond Pavlovian biases in responses and RTs, we also found cue valence to affect gaze position: During the cue presentation, participants' gaze showed less dispersion from the center of the screen for Avoid cues compared to Win cues in a time range around 200–280 ms after cue onset, with differences becoming stronger with learning. This finding is reminiscent of previous findings of

"freezing of gaze" induced by a threat of shock manipulation (Merscher & Gamer, 2024; Merscher et al., 2022; Rösler & Gamer, 2019). Note however that our paradigms extends these findings: Previous studies encouraged participants to visually explore photos of natural scenes while they prepared for a button press in order to prevent an electric shock. In contrast, in our task, participants were instructed to maintain fixation at the center of the screen while an aversive cue signaling the chance of losing points was presented. Hence, we show that freezing of gaze can be observed under minimal conditions even when participants are instructed to move their eyes as little as possible and even if the "threat" merely consists in losing points. Crucially, we observed that this freezing of gaze phenomenon was not yet present on the first five occurrences of a cue when cue valence had not been learned, but emerged only in the middle of blocks when participants had become aware of the cue valence.

Our results corroborate recent evidence that freezing does not merely affect limb movements, but also the oculomotor system. Past research has shown that the chance to gain rewards speeds up saccades (Manohar et al., 2015; Shadmehr, Reppert, Summerside, Yoon, & Ahmed, 2019; Tachibana & Hikosaka, 2012), a process sensitive to dopamine and likely implemented by the direct pathway of the basal ganglia (Grogan, Sandhu, Hu, & Manohar, 2020; Kawagoe, Takikawa, & Hikosaka, 1998). Conversely, the indirect pathway in the basal ganglia seems responsible for the suppression of eye movements in presence of low-value objects (Amita & Hikosaka, 2019; Kim, Amita, & Hikosaka, 2017), a role it might also play for negative events such as aversive cues and threats of punishment. Overall, these findings suggest a more principled role of the basal ganglia in modulating the vigor of eye movements as a function of incentives (Park, Coddington, & Dudman, 2020; Turner & Desmurget, 2010). Our results contribute to this literature by showing how the oculomotor system can give insights in reward- and punishment processing not only in animals, but also in humans (Shadmehr et al., 2019).

Pupil dilation reflects effort expenditure in a graded fashion

Apart from gaze, also pupil dilations reflected aspects of the Motivational Go/NoGo Task. The biggest effect on pupil dilations was caused by responses, with much stronger pupil dilations for Go than for NoGo responses. This finding concords with a large body of literature reporting stronger pupil dilations under movement preparation, movement execution, and effort exertion (Beatty, 1982;

Bijleveld, Custers, & Aarts, 2009; da Silva Castanheira, LoParco, & Otto, 2020; Kurniawan, Grueschow, & Ruff, 2021; van der Wel & van Steenbergen, 2018; Zénon, Sidibé, & Olivier, 2014). However, it is still an open question which specific processes drive these previously observed response-related pupil dilations. They may just constitute an epiphenomenon of motor movements, i.e. an signal that qualitatively reflects whether a movement is executed or not in an all-or-nothing fashion (Richer & Beatty, 1985; Richer, Silverman, & Beatty, 1983). Alternatively, pupil dilations have been suggested to reflect the effort that is required to execute a response in a more graded, continuous fashion (da Silva Castanheira et al., 2020; van der Wel & van Steenbergen, 2018). In the current study, response-related dilations were stronger for Avoid than to Win cues, which goes against the notion of pupil dilations being a motor epiphenomenon. We propose that pupil dilations reflect that effort participants need to recruit in order to invigorate a Go response.

While Pavlovian biases facilitate Go responses under the chance of reward, they suppress responses under the threat of punishment, necessitating the voluntary recruitment of effort to overcome aversive inhibition and invigorate Go responses. Reduced responses rates, slower responses, and reduced gaze dispersion under Avoid cues reflect the global, rapid effects of aversive cues on all motor systems (Schmidt & Berke, 2017; Wessel & Aron, 2017). In order to overcome such aversive inhibition, participants first need to detect the conflict between the bias-triggered response (NoGo) and the required response (Go) and specify control demands, a process likely implemented by the anterior cingulate cortex (ACC) (Cavanagh & Frank, 2014; Cohen, 2014). Subsequently, they need to recruit mechanisms to boost the controlled, deliberate response over the automatic, bias-triggered response, likely implemented by lateral prefrontal and motor cortices as well as the basal ganglia (Cohen & Cavanagh, 2011; Shenhav, Botvinick, & Cohen, 2013; Swart et al., 2018).

Previous studies mostly focused on the inhibition of incorrect Go responses to Win cues (Cavanagh et al., 2013; Swart et al., 2018), which likely involves recruitment of the hyperdirect pathway projecting to the subthalamic nucleus and “braking” ongoing action preparation (Frank, 2006; Schmidt & Berke, 2017). Conversely, in this study, we investigated the mechanisms boosting Go responses in face of aversive inhibition, i.e., providing additional “drive” to the direct pathway releasing Go actions.

Such processes have previously been studied in the context of speeding up responses through incentives (Grogan et al., 2020; Manohar et al., 2015; Mazzoni, Hristova, & Krakauer, 2007; Turner & Desmurget, 2010). We propose that the same subcortical mechanisms can be voluntarily recruited, e.g. when instructed to prioritize speed (Muhammed, Dalmaijer, Manohar, & Husain, 2018), and can be used to turn a NoGo response (i.e., lack of drive in the basal ganglia) into a Go response, a process that requires "effort" similarly to voluntarily speeding up responses. Note however that this type of effort, which is indexed via pupil diameter, is not associated with relatively faster, but slower responses, reflecting situations where eventual Go responses result sequentially from conflict detection and subsequent effort recruitment. Hence, in the context of this task, subcortical "vigor" mechanisms might not (only) be responsible for speeding up responses, but also for executing responses in the first place.

Higher pupil dilations during responses to Avoid than to Win cues specifically reflect effort demands, which dynamically change as a function of learning. Differences between Avoid and Win cues occurred specifically in the middle of each block, i.e., after participants were made aware of the cue valence, but before they had fully learned the correct response. At the beginning of each block, new cues were introduced, and until participants had experienced a win or loss of points, they could not know the cue valence. Thus, until the aversive nature of Avoid cues had been experienced, these cues did not induce aversive inhibition nor did they motivate additional effort recruitment. Similarly, little effort was required at the end of blocks when the instrumental learning system had acquired reliable action values that were unlikely to be "swayed" by Pavlovian biases (Dorfman & Gershman, 2019). Additionally, at the end of each block, the experienced rate of punishments had become lower due to increased accuracy, which in turn might have lowered the aversive value of the cues and reduced aversive inhibition. In summary, effort was recruited only after the aversive nature of cues had become clear, but only until responses to them became well-learned, concurring with the interpretation of pupil dilation as reflecting effort recruited to overcome aversive inhibition.

A final piece of evidence suggesting that pupil dilations reflect effort recruitment in a continuous fashion is the finding that dilations were stronger for slower compared to faster responses. Slow responses are often interpreted as reflecting action selection against difficulties, involving

effortful cognitive control to resolve conflict (Cavanagh & Frank, 2014; Cohen, 2014; Frank, 2006). The link between dilations and responses was particularly strong for incorrect Go responses (to NoGo cues), which were slower than correct responses (to Go cues), implying that these do not reflect "impulsive" errors, but rather deliberate choices made in spite of previous feedback providing evidence against Go responses. Such slow, incorrect responses might have required particularly high levels of effort to trigger a Go response against competing instrumental processes suggesting a NoGo response. Taken together, action-related modulations of the pupil response are likely not mere artifacts of executing an action, but reflect how much effort has to be recruited to successfully execute an action.

Putative neural mechanisms of aversive biases and their suppression

Past studies on bodily freezing have focused on effector systems other than the oculomotor system and highlighted the role of subcortical areas outside the basal ganglia (Evans, Stempel, Vale, & Branco, 2019; Roelofs, 2017; Roelofs & Dayan, 2022). Bodily freezing is likely implemented by the amygdala and the periaqueductal grey, while the subsequent switch to action is implemented by the perigenual anterior cingulate cortex (pgACC) inhibiting the amygdala (Hashemi et al., 2019). A large body of literature has found neutral activity in the ACC to reflect the level of physical or cognitive effort exerted (Klein-Flügge, Kennerley, Friston, & Bestmann, 2016; Skvortsova, Palminteri, & Pessiglione, 2014; Vassena et al., 2014) and to correlate with pupil size (Ebitz & Platt, 2015; Joshi, Li, Kalwani, & Gold, 2016; Muller, Mars, Behrens, & O'Reilly, 2019; O'Reilly et al., 2013). In the context of our task, it is possible that processes in ACC evaluate whether to recruit effort to overcome aversive inhibition and subsequently suppress processes in the amygdala that are responsible for the freezing response. These events might require noradrenergic input, which is visible in pupil diameter.

A large body of previous literature has assigned a monitoring role to noradrenaline, i.e., to encode the amount of unexpected uncertainty in the environment (O'Reilly et al., 2013; Yu & Dayan, 2005). The presented data do not provide further support this idea. Instead, they concur with past literature linking direct recordings of noradrenaline neurons in monkeys to effort expenditure (Bornert & Bouret, 2021; Varazzani, San-Galli, Gilardeau, & Bouret, 2015). Specifically, one study recorded activity from the substantia nigra and locus coeruleus, the primary sources of dopamine and

noradrenaline, while monkeys performed a reward/ effort trade-off task involving a grip forcer (Varazzani et al., 2015). Dopamine reflected expected value and required effort before response onset, while noradrenaline reflected the grip force actually exerted during responses, which was also reflected in pupil diameter. In line with these results, one interpretation of the presented data is that effort expenditure—via noradrenergic activation—dominates the pupillary signal (Hess & Polt, 1964; Kahneman, 1973). Of note, many situations characterized by high unexpected uncertainty require effort in order to inhibit an old response strategy and switch to a new strategy (Algermissen et al., 2019; Lavín et al., 2014; Nassar et al., 2012; O'Reilly et al., 2013; Preuschoff et al., 2011), a finding also present in our data (see Supplementary Material S04). In sum, pupil size and phasic noradrenaline might not reflect unexpected uncertainty per se, but the downstream consequences of increased effort recruited for adopting a new response strategy (Bouret & Sara, 2005).

While several studies have reported a correlation between pupil diameter and activity of the locus coeruleus, the main source of noradrenaline in the brain (Joshi & Gold, 2019; Joshi et al., 2016; Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014), this link has recently come under debate (Megemont, McBurney-Lin, & Yang, 2022). Pupil size also correlates with the trial-by-trial BOLD signal activity in other brain stem nuclei, specifically the dopaminergic ventral tegmental area and substantia nigra, at least during rest (Lloyd, de Voogd, Mäki-Marttunen, & Nieuwenhuis, 2023). It might be interesting to consider the possibility that the action-induced modulation of pupil dilation in this study in fact reflect dopaminergic activity (Varazzani et al., 2015; Walton & Bouret, 2018). In line with this hypothesis, one of our past studies (Algermissen et al., 2022) found the same pattern observed in pupil dilations in this study—a strong main effect of action, with a particular strong signal for actions to Avoid cues—in the striatal BOLD signal, which replicated previous patterns of VTA and striatal BOLD signal (Guitart-Masip et al., 2012) and was recently replicated itself (Queirazza et al., 2023). The same study found striatal BOLD to be correlated with midfrontal theta power. Other studies have found pupil diameter to be related to midfrontal theta power (Dippel, Mückschel, Ziemssen, & Beste, 2017; Lin, Saunders, Hutcherson, & Inzlicht, 2018) and the P3, an evoked potential likely generated by stimulus-locked oscillations in the theta range (de Gee, Correa, Weaver, Donner, & van Gaal, 2021;

Murphy, Robertson, Balsters, & O'Connell, 2011; Nieuwenhuis, Aston-Jones, & Cohen, 2005). In sum, striatal BOLD, midfrontal theta power, and pupil diameter might all reflect the same underlying signal, which however is not noradrenergic, but dopaminergic in nature.

Taken together, freezing induced by aversive cues is likely implemented by subcortical mechanisms involving the amygdala, periaqueductal gray, and indirect pathway of the basal ganglia. Subsequent action initiation to overcome aversive inhibition might require mechanisms in ACC that are noradrenergic and reflected in pupil size. Alternatively, one might consider the option that pupil size reflects dopaminergic processes in the striatum. The striatum evaluates whether to recruit effort or not (the "value of work") (Collins & Frank, 2014; Hamid et al., 2016; Syed et al., 2016; Westbrook, Frank, & Cools, 2021), resulting in higher activity when a Go response is emitted and particularly so when it has to be pushed through against aversive inhibition. The same signal might be visible in midfrontal theta power and the task-evoked pupil dilation. Under this perspective, the direct and indirect pathways in the basal ganglia responsible for speeding and slowing saccades might also be responsible for invigorating hand or finger movements in face of aversive inhibition.

No effects of arousal priming manipulation

In this study, we used a previously established manipulation that subliminally presented faces with angry or neutral faces to induce high vs. low arousal (Allen et al., 2016). We did not observe any effects on responses, RTs, or pupil dilation. Confidence intervals and raw data plots indicated that the effect of the manipulation on all dependent measures was close to zero (Fig. 4), with little variation across participants, providing strong evidence for a null effect. Hence, although this procedure has been used successfully in the past (and proven seemingly effective in data from four pilot participants we had collected initially), it was unsuccessful in this study. Likely, the presentation duration was too short for participants to (even subliminally) process the emotional faces. The pupillometry data in particular provides strong evidence that no processing of the emotional faces occurred. This failure to use a subliminal manipulation to induce arousal aligns with other recent reports calling into question the effectiveness of subliminal manipulations reported in the literature (Mudrik & Deouell, 2022). Several cognitive processes previously reported to occur without awareness, including emotional face

processing, might in fact require awareness (Mudrik & Deouell, 2022; Skora, Livermore, Dienes, Seth, & Scott, 2023; Vadillo, Malejka, Lee, Dienes, & Shanks, 2022). It is possible that subsets of participants who perceived stimuli supraliminally did in fact drive seemingly subliminal effects in past studies (Skora et al., 2023).

Limitations

The present study features a number of limitations and points at new directions for future research. Firstly, the unsuccessful subliminal manipulation motivates the question whether a supraliminal manipulation might be more successful. However, for supraliminally presented stimuli, even more care must be taken in matching their visual properties, and condition differences could reflect differences in low-level stimulus processing. Furthermore, consciously perceived emotional stimuli can induce high-level changes in response strategy, i.e., demand characteristics (Mahlberg et al., 2021), which necessitates the use of an elaborate and effective cover story. Lastly, the presence of strong response-related transients in the pupil data might potentially camouflage more subtle stimulus-induced effects. Other physiological measures of arousal such as heart rate and skin conductance might be more suitable to measure the effects of supraliminally presented arousing stimuli (Hashemi et al., 2019; Klaassen et al., 2021). However, these measures need much longer measurement periods, requiring a slower trial structure.

In the present data, pupil diameter peaked around 1,600 ms after stimulus onset and returned to baseline around 3,000 ms, showing a slower time course than previous studies on pupil dilation (Hoeks & Levelt, 1993) and warranting care when pre-registering analysis windows. The time course of the pupil dilation might vary considerably as a function of the task structure and should be measured in pilot data before pre-registering a definite analysis window.

Summary

In summary, our results shed new light on the effects of aversive cues on motor behavior (eye and hand movements) and on the effortful counter-mechanisms recruited to overcome aversive inhibition. Aversive cues reduced response rates, slowed responses and reduced gaze dispersion (“freezing of gaze”). Over time, participants learned to counteract this aversive Pavlovian bias and make

Go responses even to aversive cues. These responses were associated with particularly strong pupil dilations, which we interpret as reflecting additional effort recruitment in order to overcome aversive inhibition. While previous literature has primarily focused on how impulsive responding to Win cues can be suppressed (Cavanagh et al., 2013; Swart et al., 2018), this study sheds light on the opposite end of Pavlovian biases, namely how humans can invigorate responding against factors holding them back. Future studies could use pupillometry in the context of aversive inhibition to further probe this underexplored facet of cognitive control.

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Supplementary Files

Supplementary Material S01: Overview of results from all mixed-effects regression models

Here, we report an overview over all major statistical results reported in the main text and the supplementary material. For details on how mixed-effects regression were performed, see the Methods section of the main text.

Model ID	DV	IV	*b*	*SE*	$\chi^2(1)$	*p*
1	Response	Req. action	1.367	0.096	66.423	< .001
		Valence	0.537	0.100	20.986	< .001
		Req. action x valence	0.068	0.057	1.238	.246
2	RT	Req. action	-0.143	0.028	20.446	< .001
		Valence	-0.161	0.025	27.329	< .001
		Req. action x valence	-0.007	0.023	0.083	.773
3	Response	Req. action	1.368	0.097	66.422	< .001
		Valence	0.539	0.101	20.957	< .001
		Manipulation	-0.008	0.028	0.054	.816
		Req. action x valence	0.068	0.058	1.321	.250
		Req. action x manipulation	-0.019	0.028	0.319	.573
		Valence x manipulation	0.006	0.030	0.034	.854
		Req. action x valence x manipulation	-0.014	0.029	0.170	.680
4	RT	Req. action	-0.141	0.028	26.046	< .001
		Valence	-0.159	0.025	40.344	< .001
		Manipulation	-0.005	0.017	0.080	.777
		Req. action x valence	-0.009	0.023	0.152	.697
		Req. action x manipulation	0.014	0.017	0.713	.398
		Valence x manipulation	0.008	0.018	0.211	.646
		Req. action x valence x manipulation	-0.025	0.016	2.477	.116
5	Response	Req. action	1.379	0.096	67.271	< .001
		Valence	0.560	0.101	21.971	< .001
		Dilation	0.309	0.054	22.519	< .001
		Req. action x valence	0.091	0.059	2.246	.134
		Req. action x dilation	-0.119	0.036	7.945	.005
		Valence x dilation	-0.004	0.041	0.009	.924
		Req. action x valence x dilation	-0.012	0.042	0.065	.799
6	RT	Req. action	-0.144	0.027	21.532	< .001
		Valence	-0.146	0.025	23.429	< .001
		Dilation	0.096	0.017	43.879	< .001
		Req. action x valence	-0.013	0.023	0.305	.580
		Req. action x dilation	0.039	0.017	5.338	.021
		Valence x dilation	-0.034	0.018	3.140	.076
		Req. action x valence x dilation	0.004	0.017	0.057	.812
7	Response	Req. action	1.386	0.096	67.406	< .001
		Valence	0.563	0.101	22.201	< .001
		Manipulation	0.013	0.030	0.154	.695d
		Dilation	0.327	0.053	25.649	< .001
		Req. action x valence	0.090	0.059	2.121	.145
		Req. action x manipulation	-0.014	0.031	0.123	.726
		Valence x manipulation	0.018	0.031	0.259	.611
		Req. action x dilation	-0.109	0.038	5.907	.015
		valence x dilation	-0.003	0.042	0.021	.886
		Manipulation x dilation	0.024	0.033	0.370	.543

		Req. action x valence x manipulation	-0.011	0.032	0.087	.768
		Req. action x valence x dilation	-0.001	0.044	0.020	.887
		Req. action x manipulation x dilation	0.023	0.033	0.360	.549
		Valence x manipulation x dilation	0.001	0.033	0.019	.891
		Req. action x valence x manipulation x dilation	0.027	0.036	0.420	.517
8	RT	Req. action	-0.145	0.027	22.266	< .001
		Valence	-0.146	0.025	24.679	< .001
		Manipulation	-0.008	0.017	0.230	.631
		Dilation	0.093	0.018	19.654	< .001
		Req. action x valence	-0.012	0.023	0.287	.592
		Req. action x manipulation	0.018	0.017	0.998	.318
		valence x manipulation	0.010	0.017	0.316	.574
		Req. action x dilation	0.041	0.017	5.476	.019
		valence x dilation	-0.033	0.018	2.979	.084
		Manipulation x dilation	0.011	0.016	0.509	.475
		Req. action x valence x manipulation	-0.032	0.016	3.661	.056
		Req. action x valence x dilation	0.003	0.017	0.019	.891
		Req. action x manipulation x dilation	-0.024	0.017	1.867	.172
		Valence x manipulation x dilation	-0.031	0.019	2.452	.117
		Req. action x valence x manipulation x dilation	0.024	0.016	3.1817	.051

Table S01. Overview of the results from all mixed-effects logistic and linear regression models reported in the main text of the manuscript.

Supplementary Material S02: Overview of means and standard deviations of responses and RTs per task condition

Responses

Req. Act.	Go	Go	NoGo	NoGo
Valence	Win	Avoid	Win	Avoid
Mean	0.875	0.759	0.410	0.216
SD	0.124	0.122	0.258	0.096

Table S02. Means and standard deviations of Go/NoGo responses across participants per required action x valence condition.

Responses

Req. Act.	Go	Go	Go	Go	NoGo	NoGo	NoGo	NoGo
Valence	Win	Win	Avoid	Avoid	Win	Win	Avoid	Avoid
Prime	High	Low	High	Low	High	Low	High	Low
Mean	0.871	0.880	0.754	0.763	0.414	0.405	0.215	0.217
SD	0.131	0.124	0.138	0.124	0.258	0.269	0.106	0.102

Table S03. Means and standard deviations of Go/NoGo responses across participants per required action x valence x prime condition.

RTs

Req. Act.	Go	Go	NoGo	NoGo
Valence	Win	Avoid	Win	Avoid
Mean	0.641	0.707	0.707	0.756
SD	0.071	0.076	0.122	0.103

Table S04. Means and standard deviations of reaction times across participants per required action x valence condition.

RTs

Req. Act.	Go	Go	Go	Go	NoGo	NoGo	NoGo	NoGo
Valence	Win	Win	Avoid	Avoid	Win	Win	Avoid	Avoid
Prime	High	Low	High	Low	High	Low	High	Low
Mean	0.641	0.641	0.713	0.702	0.711	0.704	0.738	0.771
SD	0.081	0.067	0.078	0.083	0.131	0.123	0.131	0.116

Table S05. Means and standard deviations of reaction times across participants per required action x valence x prime condition.

Supplementary Material S03: Correlations of the effects of cue valence, arousal manipulation, and trial-by-trial pupil dilation on responses and RTs with questionnaires

In line with the exploratory analysis plans in mentioned in our pre-registration, we extracted the per-participant coefficients (fixed plus random effects) for (a) the effect of cue valence on responses and RTs (Pavlovian bias), (b) the effect of the arousal manipulation on responses and RTs, and (c) the effect of pupil dilation on responses and RTs. We then computed correlations of these coefficients with trait anxiety (STAI, Form Y-2, 20 items) (Spielberger, Gorssuch, Lushene, Vagg, & Jacobs, 1983) and the five sub-scales negative urgency, lack of perseveration, lack of premeditation, sensation seeking, and positive urgency of the UPPS-P Impulsive Behavior Scale (short version, 20 items) (Cyders, Littlefield, Coffey, & Karyadi, 2014) One might plausibly hypothesize that trait anxiety would be associated with a stronger effect of the exogenously induced arousal on responses and RTs, and/or with a stronger effect of endogenous arousal fluctuations as reflected in trial-by-trial pupil diameter on responses and RTs. Furthermore, one might plausibly hypothesize that impulsivity is related to the Pavlovian bias since many impulsive behaviors can be conceptualized as automatic, cue-triggered behaviors.

See Figures S01, S02, and S03 for scatterplots of all bivariate associations. The only correlation significant at a level of $\alpha = .05$ (uncorrected) was between trait anxiety and the effect of dilations on RTs, with more anxious individuals showing a weaker link between trial-by-trial pupil dilation (supposedly reflecting fluctuations in endogenous arousal) and RTs. None of the other correlations were significant, providing no evidence for the strength of the Pavlovian bias or the effect of exogeneous or endogenous arousal on responses and RTs being related to either trait anxiety or sub-facets of impulsivity. Note that these analysis are underpowered to detect correlations of small-to-moderate size: With $N = 35$, we have 80% power to detect correlations of $|r| > 0.45$, and only correlations of $|r| > 0.33$ (50% power) will become significant.

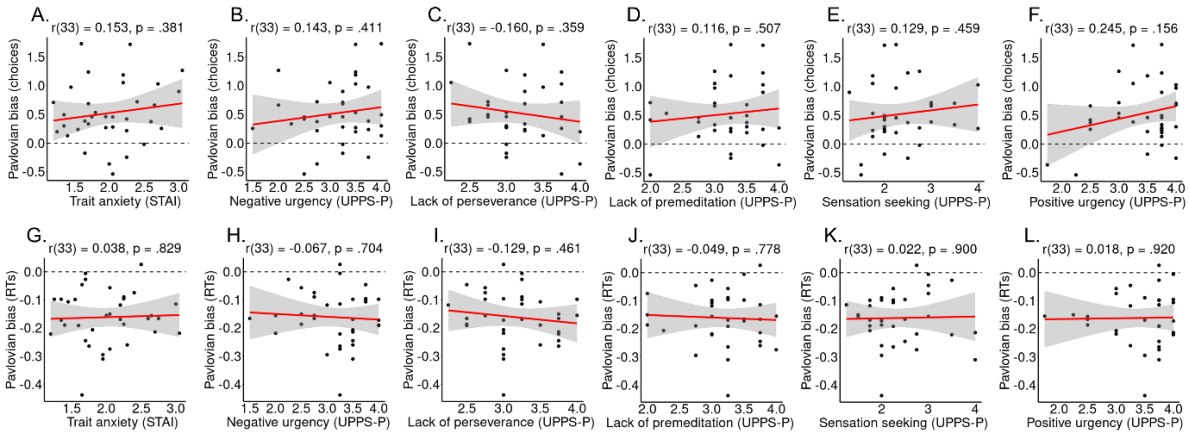

Figure S01. Association of trait anxiety and various sub-facets of trait impulsivity with the effect of valence on responses and RTs. Correlations between the effect of valence on responses (**A–F**) and on RTs (**G-L**), reflecting Pavlovian biases, and the trait anxiety (**A**, **G**) negative urgency (**B, H**), lack of perseverance (**C, I**), lack of premeditation (**D, J**), sensation seeking (**E, K**), and positive urgency (**F, L**). Black dots represent per-participant scores, the red line the best-fitting regression line, the grey shade the 95%-confidence interval. None of the displayed correlations is significant at α = .05.

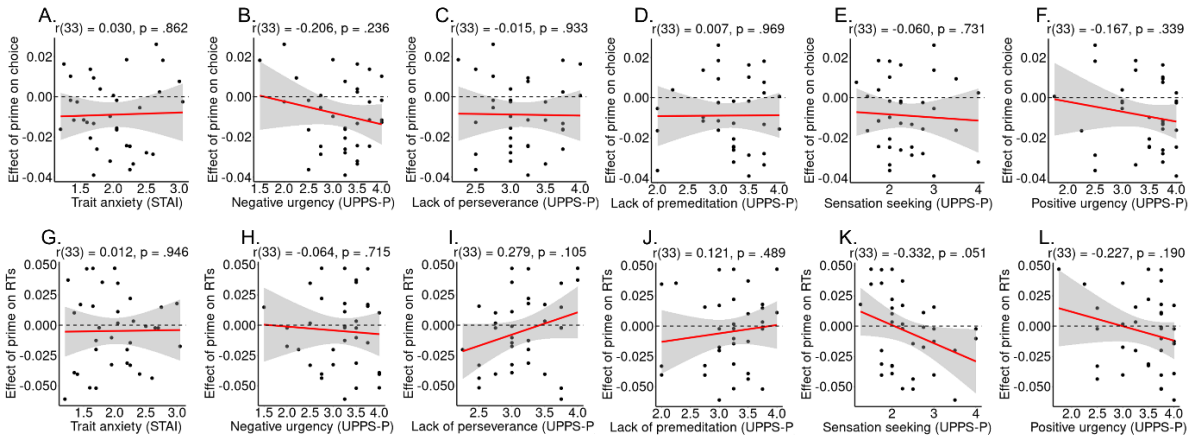

Figure S02. Association of trait anxiety and various sub-facets of trait impulsivity with the effect of the arousal manipulation on responses on RTs. Correlations between the effect of the subliminal arousal manipulation on responses (**A–F**) and on RTs (**G-L**), and the trait anxiety (**A**, **G**) negative urgency (**B, H**), lack of perseverance (**C, I**), lack of premeditation (**D, J**), sensation seeking (**E, K**), and positive urgency (**F, L**). Black dots represent per-participant scores, the red line the best-fitting regression line, the grey shade the 95%-confidence interval. None of the displayed correlations is significant at α = .05.

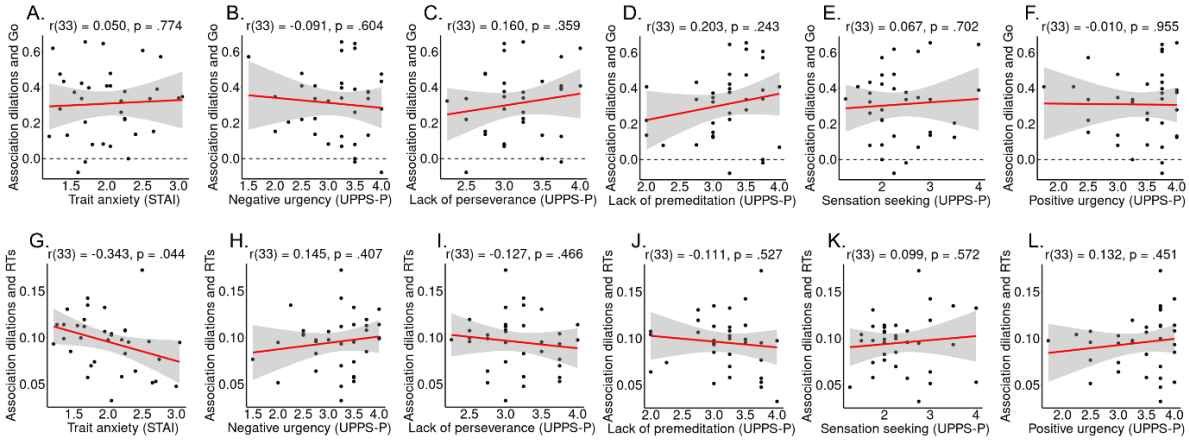

Figure S03. Association of trait anxiety and various sub-facets of trait impulsivity with the effect of trial-by-trial pupil dilation on responses on RTs. Correlations between the effect of trial-by-trial pupil dilation on responses (**A–F**) and on RTs (**G-L**), and the trait anxiety (**A**, **G**) negative urgency (**B, H**), lack of perseverance (**C, I**), lack of premeditation (**D, J**), sensation seeking (**E, K**), and positive urgency (**F, L**). Black dots represent per-participant scores, the red line the best-fitting regression line, the grey shade the 95%-confidence interval. The only correlation significant at a level of α = .05 (uncorrected) is between trait anxiety and the effect of dilations on RTs, with more anxious individuals showing a weaker link between trial-by-trial pupil dilation (supposedly reflecting fluctuations in endogenous arousal) and RTs.

Supplementary Material S04: Higher pupil dilations for response to Avoid cues than to Win cues while controlling for accuracy, RTs, and response repetition over time

We performed control analyses testing whether the difference in pupil dilation between Go responses to Avoid compared to Win cues could be due to other factors associated with increased pupil dilations, specifically (a) correct vs. incorrect responses, (b) fast vs. slow responses (median split), and (c) response repetitions vs. switches to the alternative response option (with respect to the last encounter of the same cue).

See Table S06 for inferential statistics from mixed-effects linear regression models regressing trial-by-trial pupil dilations onto accuracy, response speed, and response repetition, separately and in interaction with the performed response (Go vs. NoGo). See Table S07 for inferential statistics from generalized additive models testing whether condition differences occurred selectively at particularly time points within blocks. Incorrect responses were associated with significantly larger dilations compared to correct responses, an effect that was marginally stronger for NoGo responses (Fig. S04A). Over the time course of blocks, dilations were higher for incorrect NoGo responses than correct NoGo responses on cue repetitions 4 until 13, with no difference between incorrect and correct Go responses (Fig. S04D). Furthermore, slow responses were associated significantly with higher dilations compared to fast responses (Fig S04B; note that on NoGo trials, no RTs can be observed) throughout blocks (Fig. S04E). Lastly, trials on which participants switched their response with respect to the last encounter of the same cue were associated with significantly higher pupil dilations (Fig. S04C) throughout a block (Fig. S04F), with no interaction with the performed response. In sum, incorrect responses, slower responses, and response switches were associated with stronger pupil dilations.

Both incorrect and slower responses were associated with significantly increased pupil dilations, but also with each other: incorrect responses (to NoGo cues) tended to be slower than (correct) responses (to Go cues; see Fig. 2E, F in main text). We thus split trials with Go responses by both accuracy (correct/ incorrect) and response speed (fast/ slow; median split performed separately for correct and incorrect

responses for each participant) and tested whether both factors contributed independently to pupil dilations. Slower responses were associated with stronger dilations than faster responses irrespective of accuracy, while accuracy alone had no effect on dilations when controlling for response speed (Fig. S05 and inferential statistics in Tables S06 and S07). Hence, stronger pupil dilations on incorrect compared to correct responses follow from the former being slower than the latter. Note that GAMMs control for any changes in overall response speed or accuracy over time; the difference between fast and slow responses cannot be accounted for by increases in speed and accuracy over time.

Next, we investigated whether higher pupil dilations for Go responses to Avoid cues compared to Win cues were still observed for separate levels of accuracy, response speed (fast/ slow; median split performed separately for Win and Avoid cues for each participant), and response repetition. Dilations were still marginally significantly higher for response to Avoid cues than to Win cues irrespective of accuracy (Fig. S06A, Table S06). Additive models suggested significantly higher dilations for correct Go responses to Avoid than to Win cues on cue repetitions 4–13 as well as higher dilations for incorrect Go responses to Avoid than to Win cues on cue repetitions 6–16 (Fig. S06D, Table S07). Furthermore, while linear regression models suggested significantly higher dilations for slow than fast responses (median split performed separately for Win and Avoid cues), with no significant difference between Avoid and Win cues (Fig. S06B, Table S06), additive models suggested significantly higher dilations for slow responses to Avoid cues than slow responses to Win cues on cue repetitions 4–14, with no such difference for fast responses (Fig. S06E, Table S07). Lastly, while linear regression models indicated significantly higher dilations for response switches than response repetitions, with no differences between Avoid and Win cues (Fig. S06C, Table S06), additive models indicated that significantly higher dilations for response repetitions to Avoid than to Win cues on cue repetitions 3–13 (Fig. S06F, Table S07). For response switches, the pattern of differences was more complicated, with higher dilations for response switches for Avoid cues than for Win cues on the first three repetitions, but the reverse pattern on cue repetitions 6–13.

Taken together, these results suggest that dilations were indeed higher for Go responses to Avoid cues (for which participants had to overcome aversive inhibition) than Go responses to Win cues irrespective of accuracy, suggesting that the observed increase in pupil dilations cannot be attributed to

error processing. In fact, seemingly higher dilations to incorrect compared to correct responses are probably attributable to incorrect responses being relatively slower. Moreover, dilations were higher for Go responses to Avoid than to Win cues, but only for slow responses, with no such difference for fast responses. This pattern is in line with our interpretation of pupil dilation reflecting cognitive conflict and heightened effort recruitment in order to overcome aversive inhibition, a pattern that should lead to (and should only be observable on trials with) slow responses. In contrast, for fast responses, no such conflict might have occurred, potentially because these responses were made more “impulsively” and without proper processing of the cue or because responses had started to become well learned. Lastly, dilations on Go response repetitions (the large majority of responses) were higher for Avoid cues than Win cues, suggesting that this pattern was not induced by a different pattern of response switches for Avoid than Win cues. Notably, this pattern reversed for response switches. Note however that response switches towards Go were overall rare, and especially so for Win cues (i.e. the green dashed line in Fig. S06F reflects pupil dilations on those trials on which participants had previously performed a NoGo response to a Win cue and then decided to switch towards a Go response, likely because they deemed the previous response to be incorrect—a pattern that occurred very rarely in this task given that participants performed few NoGo responses to Win cues in the first place). In sum, these results are in line with our interpretation of heightened dilations for response to Avoid cues reflecting heightened effort recruitment in order to overcome aversive inhibition, a pattern associated with slow responses.

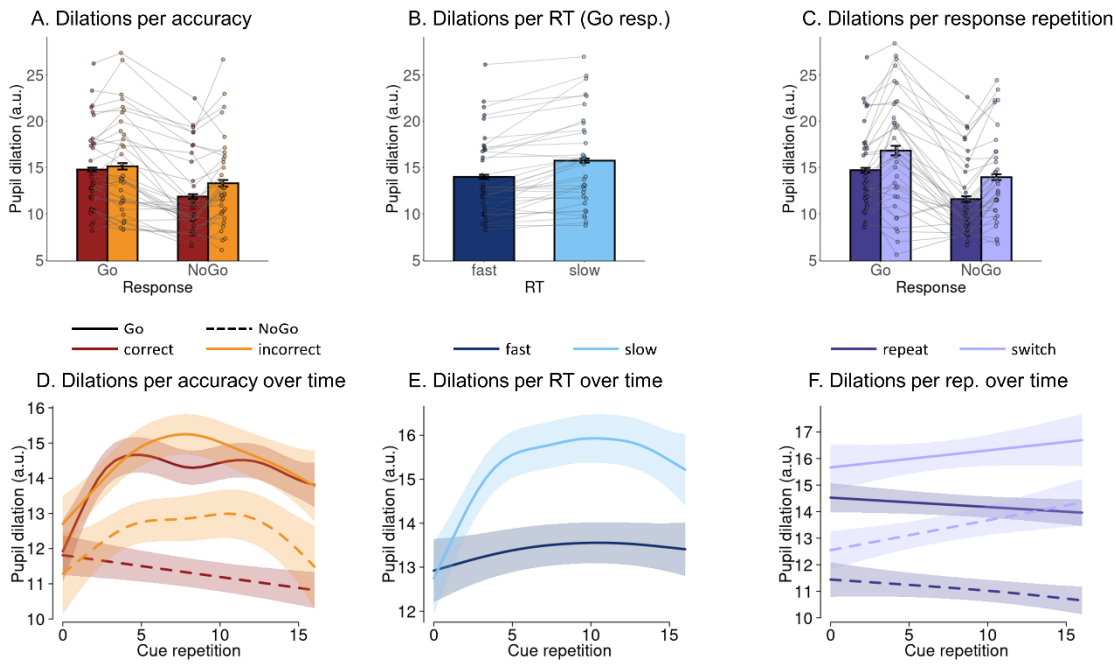

Figure S04. Association of pupil dilation with accuracy, response speed, and response repetition. **A.** Mean pupil dilation per response and accuracy (whiskers are ±SEM across participants, dots indicate individual participants). Dilations are significantly higher for Go than NoGo responses and higher for incorrect than correct responses (an effect that is marginally stronger for NoGo than Go responses). **B**. Mean pupil dilation per response speed (fast/ slow). Dilations are significantly higher for slow compared to fast responses. **C**. Mean pupil dilation per response and response repetition. Dilations are significantly higher for Go than NoGo responses and higher for response switches than response repetitions. **D**. Time course of dilations over cue repetitions (mean ± SE) as predicted from a generalized additive mixed-effects model (GAMM), separated by response and accuracy. Dilations are significantly stronger on trials with Go responses than on trials with NoGo responses throughout blocks. Furthermore, dilations are higher for incorrect than correct NoGo responses on repetitions 4–13. **E**. Time course of dilations over cue repetitions separated by response speed. Dilations are higher for slow compared to fast Go responses throughout blocks. **F**. Time course of dilations over cue repetitions separated by response and response repetition. Dilations are significantly stronger on trials with Go responses than on trials with NoGo responses and for response switches compared to response repetitions throughout blocks.

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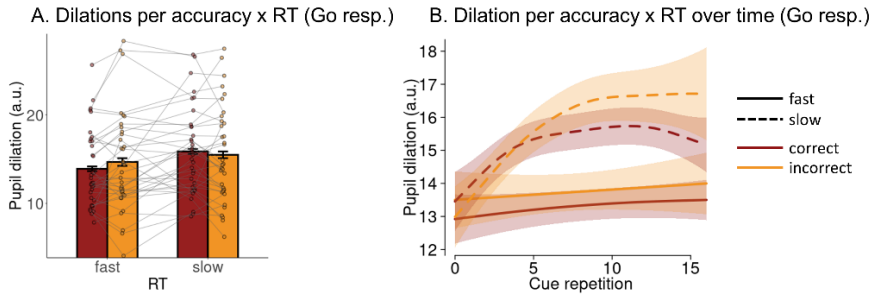

Figure S05. Association of pupil dilation with accuracy and response speed. **A.** Mean pupil dilation split by response speed and accuracy (whiskers are ±SEM across participants, dots indicate individual participants). Dilations are significantly higher on trials with slow responses than on trials with fast responses, with no significant differences between correct and incorrect responses. **B**. Time course of dilations over cue repetitions (mean ± SE) as predicted from a generalized additive mixed-effects model (GAMM), separated by accuracy and response speed. Dilations are significantly higher on trials with slow responses than on trials with fast responses, with no significant differences between correct and incorrect responses.

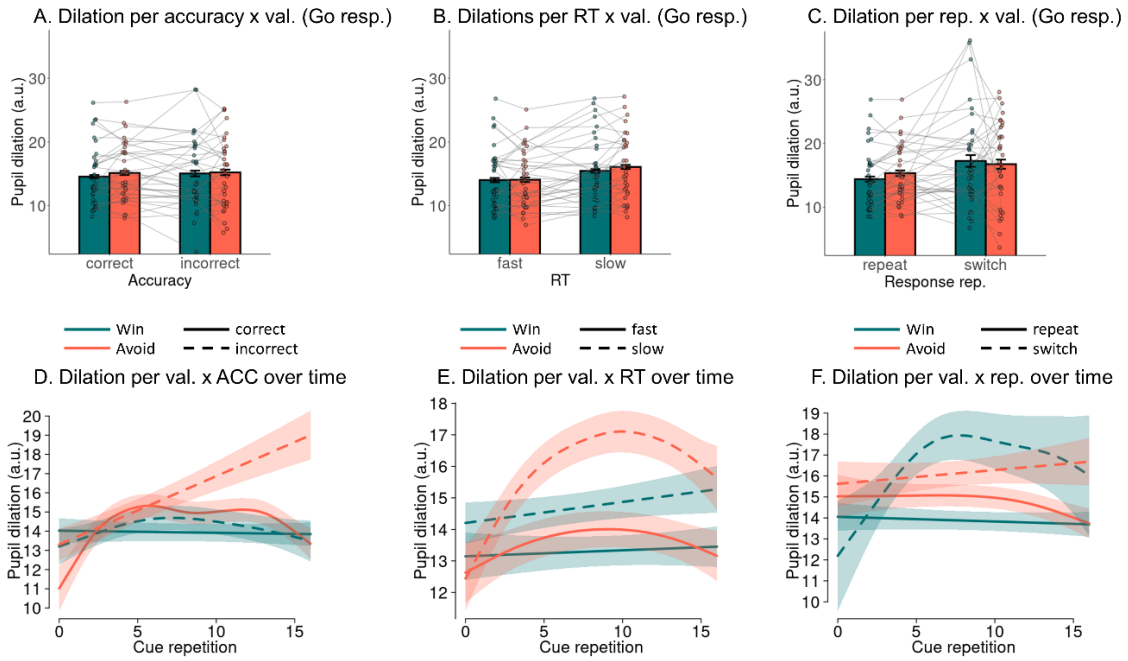

Figure S06. Higher pupil dilation for responses to Win compared to Avoid cues for trials split by accuracy, response speed, and response repetition. **A.** Mean pupil dilation on trials with Go responses per accuracy level per cue valence (whiskers are ±SEM across participants, dots indicate individual participants). Dilations are marginally significantly higher for responses to Avoid than to Win cues. **B**. Mean pupil dilation per response speed (fast/ slow) per cue valence. Dilations are significantly higher for slow compared to fast responses, while the effect of cue valence is not significant. **C**. Mean pupil dilation on trials with Go responses per response repetition per cue valence. Dilations are significantly higher for response repetitions to Avoid than to Win cues, while this effect is reversed for response switches. **D**. Time course of dilations over cue repetitions (mean ± SE) as predicted from a generalized additive mixed-effects model (GAMM), separated by accuracy and cue valence. Dilations are significantly stronger on for correct Go responses to Avoid than to Win cues on cue repetitions 4–13. Moreover, dilations are significantly stronger for incorrect Go responses to Avoid than to Win cues on cue repetitions 6–16. **E**. Time course of dilations over cue repetitions separated by response speed and cue valence. Dilations are significantly higher for slow compared to fast responses throughout blocks. Furthermore, dilations are significantly higher for slow responses to Avoid cues than to Win cues on cue repetitions 4–14, with no such difference for fast responses. **F**. Time course of dilations over cue repetitions separated by response repetition and cue valence. Dilations are significantly higher for response repetitions to Avoid than to Win cues on cue repetitions 3–13. Finally, dilations for response switches for Avoid cues are significantly higher than for Win cues on the first three repetitions, but this pattern reverses later, with stronger dilations for switches for Win cues than for Avoid cues on cue repetitions 6–13.

Model ID	Trial subset	DV	IV	*b*	*SE*	$\chi^2(1)$	*p*
1	All trials	Dilations	Accuracy (correct/ incorrect)	-0.039	0.012	8.267	.004
			Response (Go/ NoGo)	0.112	0.015	33.973	< .001
			Accuracy x Response	0.026	0.012	3.532	.060
2	Go responses	Dilations	RTs (fast/ slow)	-0.081	0.015	21.760	< .001
3	All trials	Dilations	Response repetition (repeat/ switch)	-0.105	0.019	22.924	< .001
			Response (Go/ NoGo)	0.139	0.019	34.249	< .001
			Response repetition x response	0.008	0.015	0.320	.571
4	Go responses	Dilations	Accuracy (correct/ incorrect)	-0.007	0.016	0.224	.636
			RTs (fast/ slow)	-0.073	0.017	14.429	< .001
			Accuracy x RTs	-0.018	0.016	1.386	.239
5	Go responses	Dilations	Accuracy (correct/ incorrect)	-0.017	0.017	1.099	.338
			Valence (Win/ Avoid)	-0.029	0.016	3.381	.071
			Accuracy x Valence	0.004	0.017	0.078	.730
6	Go responses	Dilations	RTs (fast/ slow)	-0.082	0.015	20.826	< .001
			Valence (Win/ Avoid)	-0.016	0.014	0.732	.392
			RTs x Valence	0.016	0.014	0.812	.368
7	Go responses	Dilations	Response repetition (repeat/ switch)	-0.107	0.027	12.841	< .001
			Valence (Win/ Avoid)	0.005	0.027	0.039	.844
			Response repetition x valence	-0.044	0.031	2.046	.153

Table S06. Results from mixed-effects linear regression models with trial-by-trial pupil dilation as dependent variable.

Model	**Parametric coefficient (Intercept difference)**	**Smooth (non-linear differences)**	**Windows of significant differences**
Accuracy *(all trials):*			
Go correct – Go incorrect	$t(5.870, 7.707) = 1.657$, $p = .098$	$F(1.001, 1.001) = 0.457$, $p = .499$	none
NoGo correct – NoGo incorrect	$t(4.460, 6.596) = 2.671$, $p = .008$	$F(3.573, 4.409) = 1.397$, $p = .198$	4 – 13
RTs *(Go responses):*			
Fast – slow	$t(5.710, 7.650) = 7.184$, $p < .001$	$F(1.422, 1.702) = 0.751$, $p = .364$	1 – 16
Repetition *(all trials):*			
Go repeat – Go switch	$t(6.054, 7.759) = 5.026$, $p < .001$	$F(1.000, 1.000) = 1.792$, $p = .181$	2 – 16
NoGo repeat – NoGo switch	$t(4.473, 6.606) = 5.904$, $p < .001$	$F(1.000, 1.000) = 1.823$, $p = .177$	1 – 16
Accuracy x RTs *(Go responses):*			
Slow Correct – Fast Correct	$t(5.107, 7.275) = 6.194$, $p < .001$	$F(1.000, 1.000) = 0.140$, $p = .709$	0 – 16
Slow Incorrect – Fast Incorrect	$t(3.000, 5.191) = 2.879$, $p = .004$	$F(1.000, 1.000) = 5.071$, $p = .025$	6 – 16
Fast Incorrect – Fast Correct	$t(3.970, 6.536) = 1.616$, $p = .106$	$F(1.003, 1.006) = 0.256$, $p = .617$	none
Slow Incorrect – Slow Correct	$t(6.416, 7.818) = 1.304$, $p = .192$	$F(1.000, 1.000) = 1.951$, $p = .163$	none
Accuracy x Valence *(Go responses):*			
Correct Avoid – correct Win	$t(5.182, 7.313) = 2.244$, $p = .025$	$F(4.479, 5.456) = 3.839$, $p = .001$	4 – 13
Incorrect Avoid – incorrect Win	$t(3.000, 5.253) = 2.159$, $p = .031$	$F(1.000, 1.000) = 2.573$, $p = .109$	6 – 16
RTs x Valence *(Go responses):*			
Fast Avoid – fast Win	$t(4.582, 6.825) = 0.958$, $p = .338$	$F(1.798, 2.176) = 0.408$, $p = .758$	none
Slow Avoid – slow Win	$t(5.974, 7.799) = 3.222$, $p = .001$	$F(2.384, 2.936) = 2.409$, $p = .065$	4 – 14
Repetition x Valence *(Go responses):*			
Repeat Avoid – repeat Win	$t(5.225, 7.400) = 3.246$, $p = .001$	$F(1.856, 2.278) = 0.869$, $p = .353$	3 – 13
Switch Avoid – switch Win	$t(5.710, 7.650) = 7.184$, $p < .001$	$F(1.422, 1.702) = 0.751$, $p = .364$	0 – 2, 6 – 13

Table S07. Results from generalized additive mixed models (GAMMs) with difference smooths between two conditions. The parametric term reflects a linear difference between conditions, while the smooth terms reflects any non-linear difference. Both add up to the total term. The time window of significant condition differences is automatically returned by the model. For the accuracy x RT and RT x valence models, the median split into fast and slow responses is performed separately for correct/ incorrect responses and Win/ Avoid cues for each participant.

Supplementary Material S05: Effect of arousal manipulation on the pupil time course within and across trials

To test for any effect of the arousal manipulation on pupil dilation at any time point within a trial, we computed the raw pupil time course per condition (high vs. low arousal) for every participant and then the average per condition across participants. A cluster-based permutation test yielded no significant difference at any time point (no cluster above the cluster-forming threshold of $|t| > 2$), suggesting again no effect of the arousal manipulation on pupil dilation (Fig. S07A).

Furthermore, we tested whether the arousal manipulation affected pupil dilations at any time point within a block using generalized additive mixed-effects models. There was no difference in the trial-by-trial time course of pupil dilations between high-arousal and low-arousal trials, linear term $t(5.75, 7.61) = 0.252$, p = .801, smooth term $F(2.42, 2.98) = 1.757$, $p = .170$, suggesting again no effect of the arousal manipulation on pupil dilation (Fig. S07B).

As a final check, we tested whether individual differences in the effects of the arousal manipulation on responses, RTs, and pupil dilation were correlated, i.e., whether only those participants who showed an effect on pupil dilation also showed an effect on behavior. For this purpose, we fit regression models with the manipulation as sole independent variable and responses, RTs, and dilations and dependent variables, extracted the per-participants coefficients (fixed + random effects), and correlated them. Neither the per-participants effects of the manipulation on dilations and responses, $r(33) = -0.202$, $p = .243$ (Fig. S07C), nor the effects on dilations and RTs, $r(33) = 0.121$, $p = .487$ (Fig. S07D), were significantly correlated, providing no evidence for systematic individual differences in the effect of the arousal manipulation of behavior and physiology.

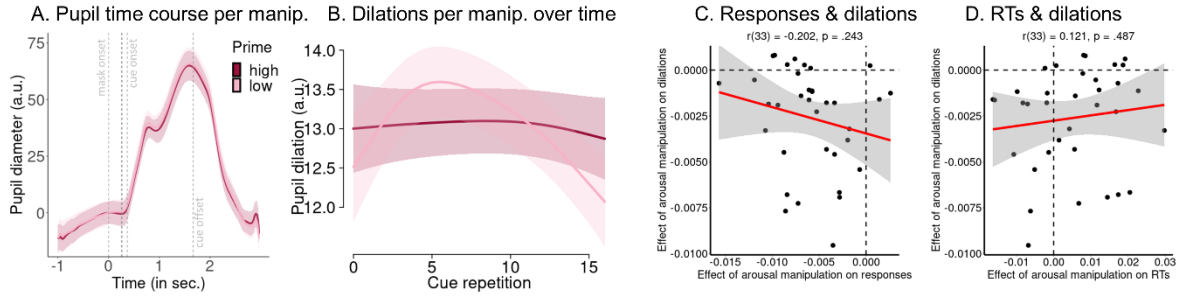

Figure S07. Effect of arousal manipulation on pupil dilation. **A.** Pupil time course within a trial (mean ± SE; baseline-corrected) separately for high vs. low arousal condition. Vertical dashed lines indicate the onset of the forward mask (at 0 ms), the prime (at 250 ms), the backwards mask (at 266 ms), the cue onset (at 366 ms), and the cue offset (at 1666 ms). There is no significant difference (no cluster above cluster-forming threshold). **B.** Time course of dilations over cue repetitions (mean ± SE) as predicted from a generalized additive mixed-effects model (GAMM), separated by arousal condition. There is no significant difference in pupil dilation between conditions at any time point. **C**. Correlation between the effect of the arousal manipulation on responses and on trial-by-trial pupil dilation. Black dots represent per-participant scores, the red line the best-fitting regression line, the grey shade the 95%-confidence interval. The correlation is not significant. **D**. Correlation between the effect of the arousal manipulation on RTs and on trial-by-trial pupil dilation. The correlation is not significant.

Supplementary Material S06: Association of pupil baseline with accuracy, RTs, and response repetition over time

Beyond task-evoked trial-by-trial pupil dilations, past literature has also investigated pre-stimulus baseline pupil diameter as a potential readout of noradrenergic activity (Aston-Jones & Cohen, 2005; Eldar, Cohen, & Niv, 2013; Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010). One the one hand, pupil baseline and task-evoked pupil dilation tend to be negatively correlated since high baseline leave less dynamic range for further dilations. In this sense, both measures could potentially capture similar phenomena and are partly redundant. However, on the other hand, pupil dilations are corrected for the immediately preceding pre-stimulus baseline and thus cannot reflect more "tonic" changes in pupil diameter on time scales longer than a single trial. In fact, pupil baseline itself tends to strongly decrease over the time course of an experiment (Muller, Mars, Behrens, & O'Reilly, 2019), likely reflecting decreases in arousal. These slower changes might reflect processes orthogonal to the trial-by-trial pupil dilations. Given that baselines are measured before cue onset, they cannot reflect the (randomized) task conditions (required action, valence, and arousal manipulation). Nonetheless, the process they reflect could still impact (or at least predict) task performance (responses, accuracy, and RTs).

While on the one hand, baseline pupil diameter could lead additional insights into cognitive processes beyond pupil dilation, on the other hand, caution is warranted given that possibility of spurious associations driven by time. When baseline pupil diameter decreases over time, any other variable that also changes on a similar time scale might be spuriously correlated with pupil diameter. Here, we used mixed-effects linear regression and generalized additive mixed effects models to test for effects of the baseline pupil diameter on responses, accuracy, and RTs (fast vs. slow, median split), controlling for potential linear and non-linear effects of time (cue repetition, 1–16).

See Table S08 for inferential statistics from mixed-effects linear regressions. See Figure S08A-C for baselines per condition averaged over trials. When ignoring time, higher baseline pupil diameter was associated with a significantly higher propensity of Go responses, incorrect responses, and slower responses (see Table S08; Fig S08A-C). The associations with accuracy and RTs disappeared when controlling for a linear effect of cue repetition (see Table S08). Most notably, additive models suggested

that baseline pupil diameter strongly decreased over time (Fig. S08D-F), with no significant difference between Go and NoGo responses, correct and incorrect responses, and only a minor (albeit significant) difference between fast and slow responses (Table S09; Fig. S08D-F) on the first eight cue repetitions, which was in fact of opposite sign (i.e., higher baselines before fast responses) to the results from the mixed-effect linear regression model (Fig. S08C). Thus, indeed, spurious associations between baseline pupil diameter and other variables arise through both changing over time, with participants showing less Go responses, less incorrect responses, and faster responses as they progress through a task block. In sum, there was strong evidence for baseline pupil diameter decreasing over the time course of a block, but no strong evidence for baseline pupil diameter affecting subsequent responses.

See Fig. S09A-C for the pupil dilation time course within a trial split by response and cue-valence when no baseline-correction is applied. Go responses to Avoid cues were associated with considerably stronger pupil dilations than Go responses to Win cues, However, this was partly driven by pre-existing baseline differences between those two trial types. Since baselines decreased with time, higher baselines on trials with Go responses to Avoid cues compared to those with Go responses to Win cues could potentially be explained by the former occurring relatively earlier within blocks (when baselines were still higher) than the latter. However, the opposite was the case: as participants learned the task, they showed more Go responses to Avoid cues with time, and the ratio between Go responses to Win and Avoid cues approached 50:50 with time. Hence, the overall decay in baseline cannot explain baseline differences between these two trial types. In fact, baseline differences were even stronger in the second half of blocks (Fig. S09C) compared to the first half (Fig. 09B), i.e. they prevailed and became even stronger as the ratio of both trial types approached 50:50. A generalized additive model corroborated that pupil baselines were significantly higher on trials with Go responses to Avoid cues compared to trials with Gon responses to Win cues in the second half of blocks (Fig. S09D, E; Table S09). In sum, Go responses to Avoid cues were not only associated with higher pupil dilations, but also higher pupil baselines, suggesting that pre-existing differences arousal before cue onset might have contributed to the mobilization of effort and invigoration of Go responses against aversive Pavlovian biases.

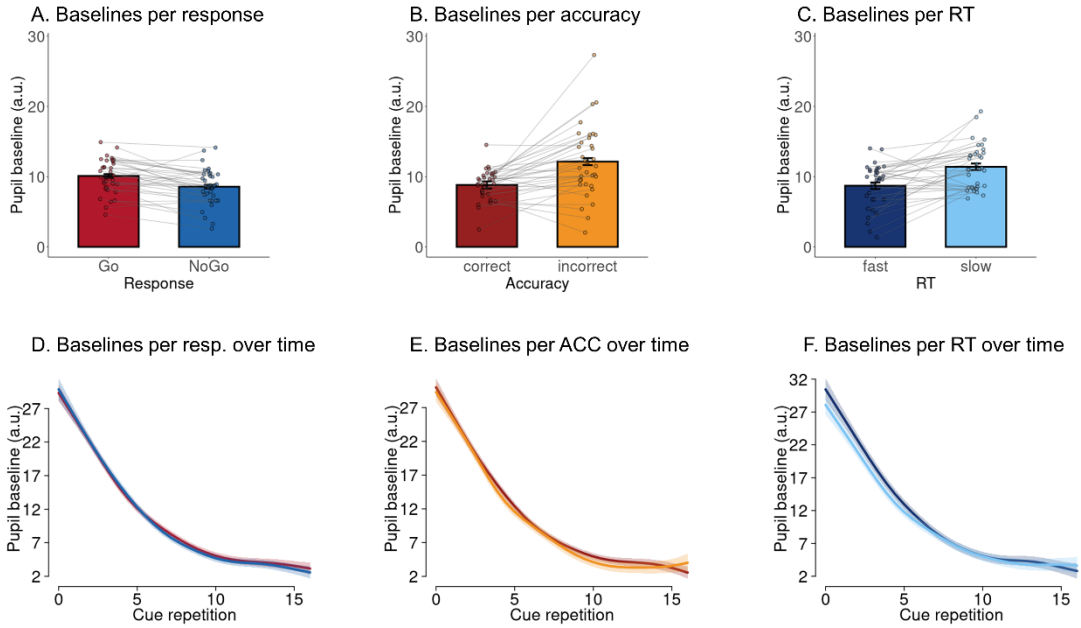

Figure S08. Relationship of pre-trial baseline pupil diameter with responses, accuracy, and RTs. **A.** Pupil pre-trial baseline split by the response made on the trial (whiskers are ± SEM across participants, dots indicate individual participants). Considering trials irrespective of their temporal position within a block, baseline pupil diameter is significantly higher before trials with Go responses than trials with NoGo responses. **B**. Pupil baseline split by the speed of the response made on the following trial (only trials with Go responses). Considering trials irrespective of their temporal position within a block, baseline pupil diameter is significantly higher before trials with incorrect responses than trials with correct responses. **C**. Pupil baseline split by the accuracy of the response made on the following trial. Considering trials irrespective of their temporal position within a block, baseline pupil diameter is significantly higher before trials with slow responses than trials with fast responses. **D**. Time course of baseline pupil diameter over cue repetitions (mean ± SE) as predicted by a generalized additive mixed-effects model (GAMM), separated by responses. There is no significant difference between trials with Go and NoGo responses. **E**. Time course of baseline pupil diameter over cue repetitions as predicted by a generalized additive mixed-effects model (GAMM), separated by accuracy. There is no significant difference between trials with Go and NoGo responses. **F.** Time course of baseline pupil diameter over cue repetitions (mean ± SE) as predicted by a generalized additive mixed-effects model (GAMM), separated by response speed (fast/ slow; median split). For the first eight cue repetitions, baseline pupil diameter is higher before fast compared to slow responses.

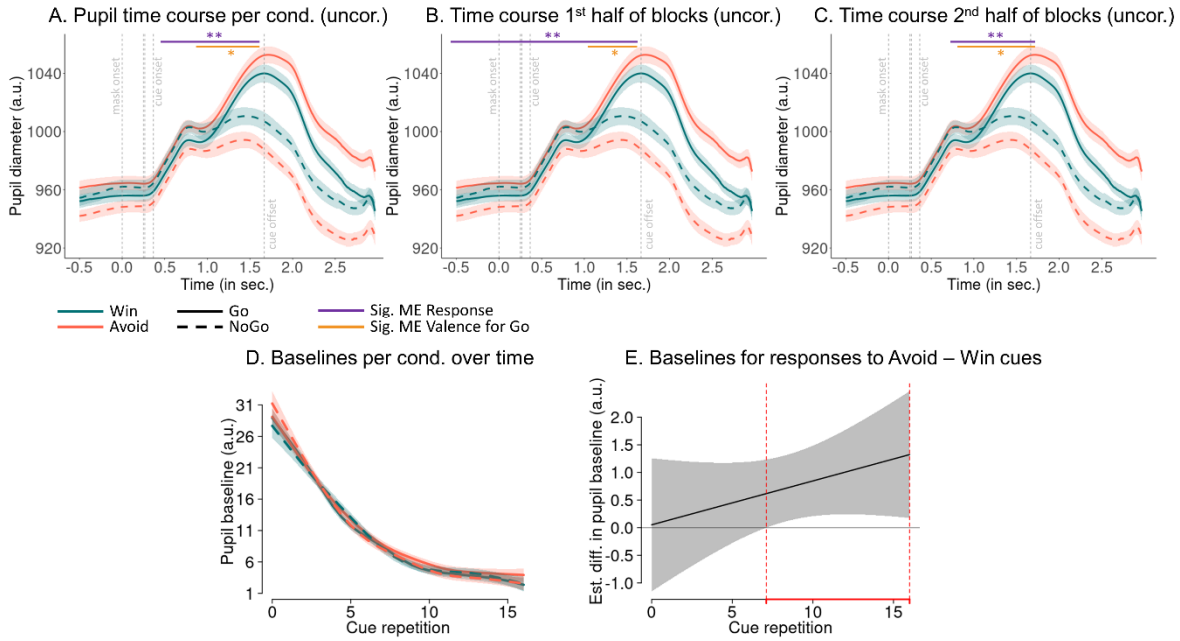

Figure S09. Pupil time course within a trial per response per cue valence without baseline correction (mean ± SEM across participants). **A.** Pupil time course split by cue valence and response made (whiskers are ± SEM across participants, dots indicate individual participants). Vertical dashed lines indicate the onset of the forward mask (at 0 ms), the prime (at 250 ms), the backwards mask (at 266 ms), the cue onset (at 366 ms), and the cue offset (at 1666 ms). The pupil dilates significantly more strongly on trials with Go responses than on trials with NoGo responses (cluster above threshold: 917–2,966 ms; $p < .001$; longer black horizontal line). Furthermore, within this time window, the pupil dilates significantly more strongly and sustainedly for responses to Avoid than to Win cues (cluster above threshold: 1,545–2,966 ms; $p = .011$; shorter black horizontal line). Note however that pre-cue pupil baselines are already higher for Go responses to Avoid cues than Go responses to Win cues. **B.** When repeating this analysis for only the first half of trials within a block, the pupil is wider on trials with Go responses than on trials with NoGo responses throughout the entire time window (cluster above threshold: -1,000–2,966 ms; $p < .001$; longer black horizontal line) and, within this time window, wider for Go responses to Avoid than to Win cues (cluster above threshold: 2,038–2,966 ms; $p = .049$; short black horizontal line). **(C)** In the second half of trials, the pupil is wider on trials with Go responses than on trials with NoGo responses in a more restricted time window (cluster above threshold: 1,137–2,966 ms; $p < .001$) and, within this time window, wider for Go responses to Avoid than to Win cues (cluster above threshold: 1,262–2,966 ms; $p < .001$). The fact that the differences in pupil diameter for Go responses to Avoid cues compared to responses to Win cues gets larger with time suggests that people learn to mobilize effort to invigorate Go responses against the Pavlovian bias (aversive inhibition) present on trials with Avoid cues. **D.** Time course of pupil baselines over cue repetitions (mean ± SE) as predicted from a generalized additive mixed-effects model (GAMM), separated by response and cue valence. Baselines are significantly stronger on trials with Go responses than on trials with Go responses to Avoid cues than trials with Go responses to Win cues from cue repetition 7 to 16, putatively reflecting that pre-cue fluctuations in arousal contribute to the invigoration of Go response against aversive Pavlovian biases. **E.** Difference line between baselines on trials with responses to Avoid cues minus Win cues. Areas highlighted in red indicate time windows with significant differences.

Model ID	DV	IV	*b*	*SE*	$\chi^2(1)$	*p*
1	Pupil baseline	Response (Go/ NoGo)	0.048	0.012	13.961	< .001
2	Pupil baseline	Accuracy (correct/ incorrect)	-0.103	0.021	18.692	< .001
3	Pupil baseline	RTs (fast/ slow)	-0.082	0.020	13.906	< .001
4	Pupil baseline	Response (Go/ NoGo)	0.023	0.010	5.336	.021
		Cue repetition (1–16, z-scored)	-0.399	0.032	60.144	< .001
		Response x cue repetition	-0.026	0.010	6.814	.009
5	Pupil baseline	Accuracy (correct/ incorrect)	0.025	0.015	2.799	.094
		Cue repetition (1–16, z-scored)	-0.429	0.034	60.685	< .001
		Accuracy x cue repetition	0.039	0.012	9.396	.002
6	Pupil baseline	RTs (fast/ slow)	0.015	0.013	0.806	.369
		Cue repetition (1–16, z-scored)	-0.422	0.033	8.646	.003
		RTs x cue repetition	0.009	0.020	0.137	.711

Table S08. Results from mixed-effects linear regression models with trial-by-trial baseline pupil diameter as dependent variable.

Model	**Parametric coefficient (Intercept difference)**	**Smooth (non-linear differences)**	**Windows of significant differences**
***Response**:*			
Go – NoGo	$t(4.777, 9.307) = -1.052$, $p = .293$	$F(1.000, 1.001) = 0.261$, $p = .616$	none
***Accuracy**:*			
Correct – incorrect	$t(4.798, 9.296) = -1.867$, $p = .062$	$F(1.129, 1.240) = 0.381$, $p = 0.73$	none
RTs:			
Fast – slow	$t(4.584, 8.867) = -1.809$, $p = .071$	$F(1.000, 1.000) = 4.700$, $p = .030$	0 – 8
Cue valence (Go responses):			
Avoid – Win	$t(4.423, 8.725) = 2.350$, $p = .019$	$F(1.000, 1.000) = 1.385$, $p = .239$	7 – 16

Table S09. Results from generalized additive mixed models (GAMMs) with difference smooths between two conditions. The parametric term reflects a linear difference between conditions, while the smooth terms reflects any non-linear difference. Both add up to the total term. The time window of significant condition differences is automatically returned by the model.

Supplementary Material S07: Outcome-locked pupil dilation

Apart from cue- (or masked-) locked pupil dilation, we also investigated outcome-locked pupil dilation (epoched from -1000 ms before until 2000 ms after outcome onset) as a function of the obtained outcome and the previously made response.

See Table S10 and Figure S10 for results from mixed-effects linear regression models as well as post-hoc *z*-tests contrasting conditions against each other. Pupil dilations were significantly stronger on trials with punishments compared to trials with rewards or neutral outcomes, while trials with rewards and neutral outcomes were not significantly different from each other. Dilations were not different between trials on which neutral outcomes signaled the absence of rewards compared to trials on which they signaled the absence of punishments.

When analyzing dilations as a function of both the obtained outcome and the previously made response, we observed main effects of outcome and response, while the interaction between them was not significant (Table S10). Pupil dilations were higher after NoGo responses compared to Go responses (Fig. S11A). However, inspection of the raw pupil time course within a trial revealed that this difference was an artifact of baseline correction: raw pupil time courses tended to be higher after Go compared to NoGo responses (for trials with punishment and neutral outcomes; Fig. S11C), leaving less dynamic range for further increases on Go compared to NoGo trials and thus leading to lower (baseline-corrected) pupil dilations on Go compared to NoGo trials (Fig. S11B).

In sum, the pupil dilated more strongly in response to punishments compared to rewards or neutral outcomes.

Model ID	DV	IV	$\chi^2(1)$	*z*	*p*
1	Pupil dilation	Outcome valence (positive/ negative)	13.439		< .001
2	Pupil dilation	Outcome displayed (reward/ neutral/ punishment)	27.237		< .001
		Punishment – neutral		6.351	< .001
		Punishment – reward		5.473	< .001
		Neutral – reward		1.093	.519
3	Pupil dilation	Outcome interpreted (rew./ no rew./ no pun./ pun.)	31.251		< .001
		Punished vs. not punished		5.591	< .001
		Punished vs. not rewarded		6.996	< .001
		Punished vs. rewarded		5.457	< .001
		Not punished vs. not rewarded		1.586	.387
		Not punished vs. rewarded		0.321	.989
		Not rewarded vs. rewarded		2.021	.180
4	Pupil dilation	Outcome displayed (reward/ neutral/ punishment)	25.704		< .001
		Response (Go/ NoGo)	19.116		< .001
		Outcome displayed x response	1.306		.521

Table S10. Results from mixed-effects linear regression models with outcome-locked trial-by-trial pupil dilation as dependent variable. Differences between any conditions were first tested with χ^2 tests and then followed up with z-tests testing two conditions against each other. *P*-values for the follow-up z-tests are corrected for multiple comparisons using the Tukey method.

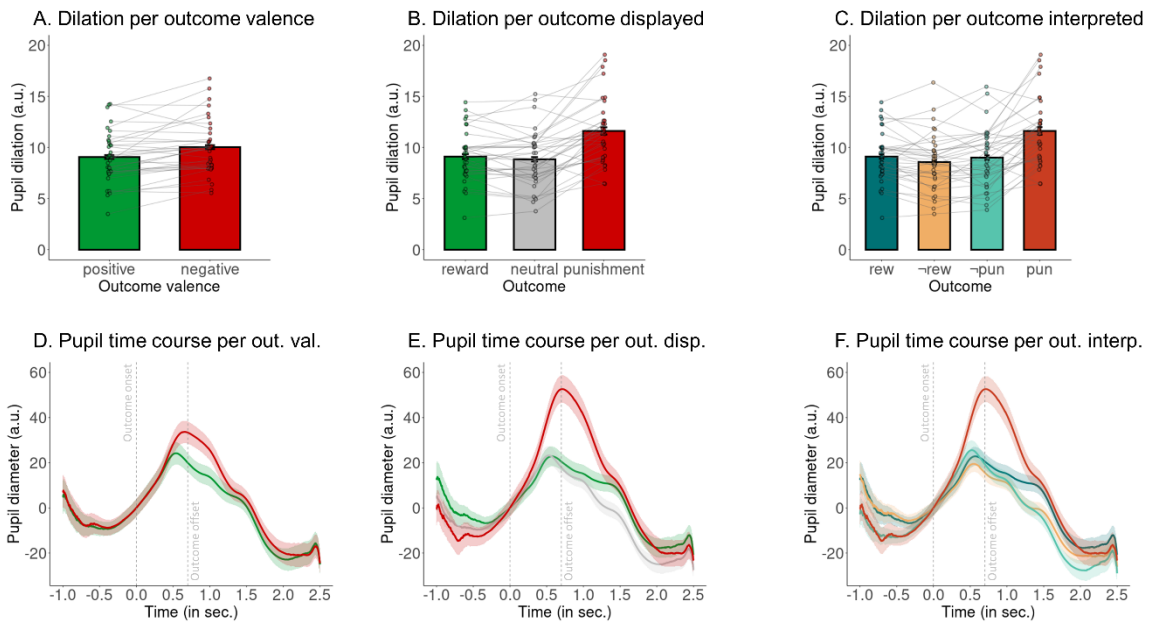

Figure S10. Effect of outcomes on outcome-locked pupil dilation. Pupil dilation as a function of outcome valence (**A**), the displayed outcome (**B**) or the outcome interpreted (with neutral outcomes recognized as signaling the absence of a reward/ punishment, **C**; whiskers are ± SEM across participants, dots indicate individual participants). The pupil dilates more strongly on trials with punishments compared to rewards or neutral outcomes. (**D-F**) Pupil time course within a trial separately for the different outcome conditions (mean ± SEM across participants; baseline-corrected). Vertical dashed line represent the onset (at 0 ms) and offset (at 700 ms) of outcomes.

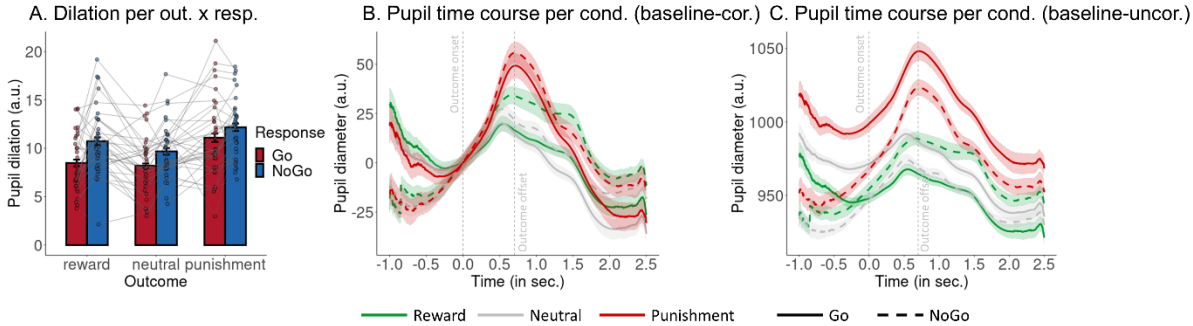

Figure S11. Effect of outcomes and responses on outcome-locked pupil dilation. **A.** Pupil dilation as a function of outcome displayed and the response performed on the same trial manipulation (whiskers are ±SEM across participants, dots indicate individual participants). When applying baseline-correction for differences in the time window of 500 ms before outcome onset, dilations are significantly higher on trials with punishments compared to trials with rewards or neutral outcomes and higher on trials with NoGo than trials with Go responses. **B**. Pupil time course within a trial separately per outcome and response condition (mean ± SEM across participants; baseline-corrected). It appears that for trials with rewards and neutral outcomes, pupil dilations are higher after NoGo than Go responses. Vertical dashed line represent the onset (at 0 ms) and offset (at 700 ms) of outcomes. **C**. Same as panel B, but not baseline corrected. It becomes clear that the pupil time course is higher after Go compared to NoGo responses, leaving less room for further increase on trials with Go compared to NoGo responses, explaining while the baseline-corrected dilations tends to be smaller after Go than NoGo responses.

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